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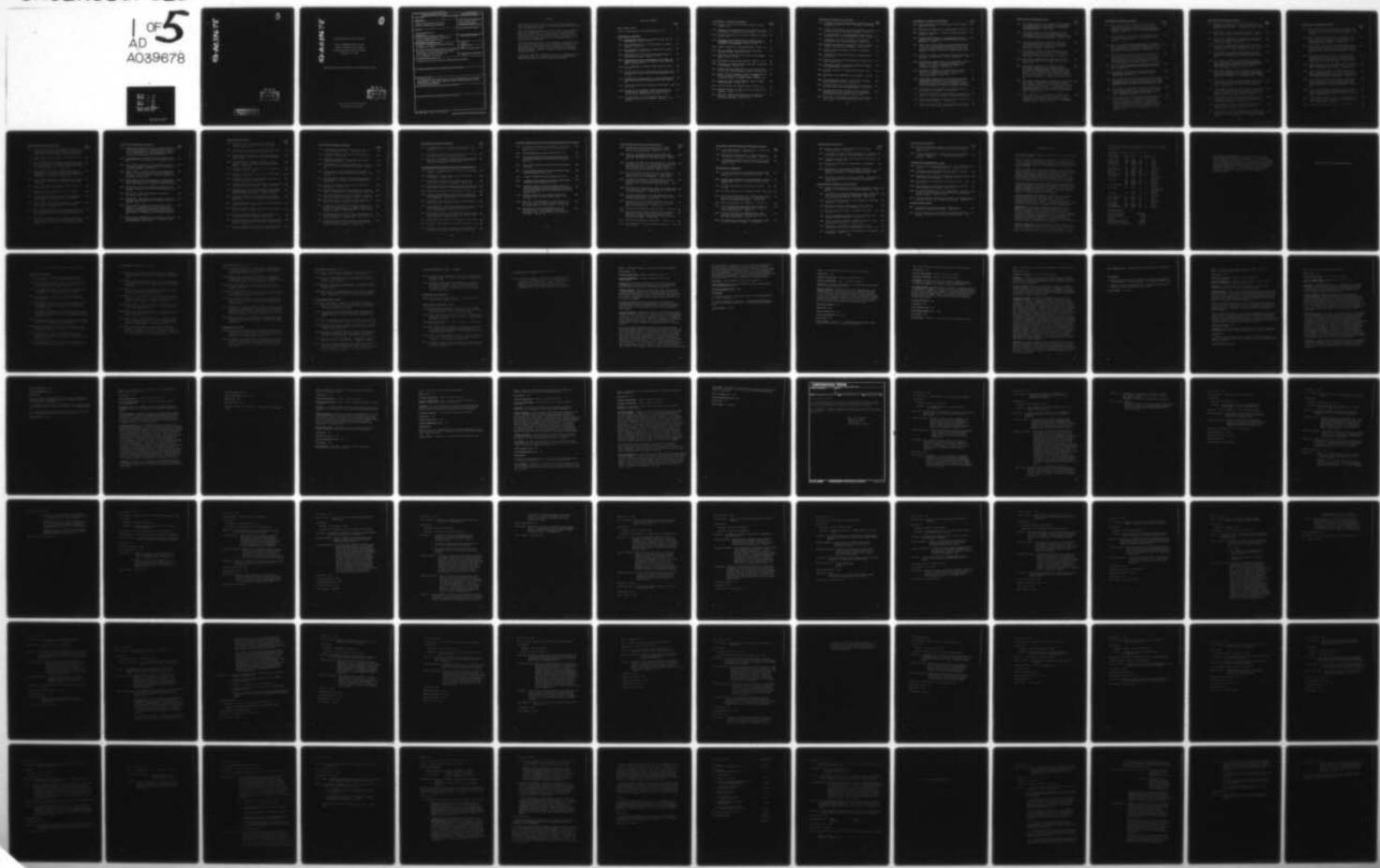
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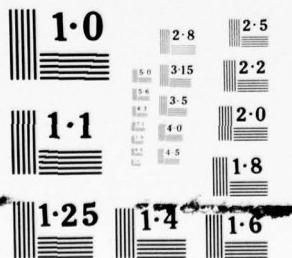
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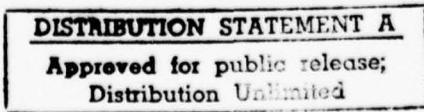
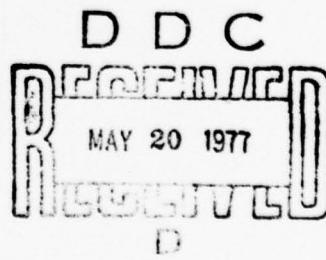
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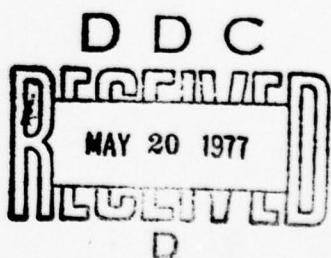
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CLINICAL INVESTIGATION SERVICE  
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Washington, D.C. 20012

This report covers the period (1 July 1975 thru 30 September 1976).



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The findings in this report are not to be construed as an official Department of Army position unless so designated by other official documents.

The investigations described in this report were conducted under the provisions of AR 40-38, Clinical Investigation Program; AR 40-7, Use of Investigational Drugs in Humans; and WR 70-1, Clinical Investigation Program, WRAMC, to insure that the rights, well being, and dignity of human subjects were maintained.

Research involving animals was performed in accordance with the "Guide for Laboratory Animal Facilities and Care" as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences - National Research Council.

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UNIT SUMMARY SHEET

This Annual Progress Report is for the period 1 July 1975 to 30 September 1976.

There were 239 investigative work units in progress at the start of FY-76. During the fifteen month period 53 were terminated, 26 work units were completed, and 117 work units were initiated. There are 216 ongoing work units at the close of FY-7T.

During February 1976, the Surgeon General instructed that three elements of the Institute of Research be reassigned to WRAMC, these elements included mission, personnel, equipment, allocated funds and assigned work areas. Of these elements the Clinical Investigation Service gained four military personnel, 12 civilian personnel, equipment listed at \$442,200, operating funds through 30 September.

During April 1976, the Surgeon General instructed that the mission, personnel and funding for Nursing Research Service assigned to WRAIR be transferred to WRAMC. This Service assumed responsibility for the equipment listed at \$9,500, funds allocated for nursing research. Personnel were assigned to Department of Nursing, WRAMC.

During July 1976, Richard Evans, COL MC, Chief, Clinical Investigation Service, assumed a dual role as Chief, Allergy-Immunology Service, and Chief, Clinical Investigation Service. Timothy M. Boehm, MAJ MC, was assigned as Asst Chief, Clinical Investigation Service, with additional clinical duties with the Kyle Metabolic Unit.

Objectives: To achieve continuous improvement in the quality of patient care. To provide experience in the mental discipline achieved by participation in such organized inquiries and to provide experience for personnel who will ultimately be teaching chiefs in military hospitals and medical specialty consultants. To maintain an atmosphere of inquiry because of the dynamic nature of health sciences. To maintain high professional standing and accreditation of advanced health programs.

Technical Approach: Provides direction and management as outlined under provision of AR 40-38, Clinical Investigation Program; AR 40-7, Use of Investigational Drugs in Humans; and WRAMC Regulation 70-1, Clinical Investigation Program, WRAMC. Provides guidance, assistance and support to the

housestaff in matters pertaining to the program. Coordinates the WRAMC program with higher headquarters and other facilities.

Manpower: Current strength is outlined.

<u>Description</u>	<u>Grade</u>	<u>MOS</u>	<u>Br</u>	<u>Actual</u>	<u>Name</u>
C, Clin Inv	O6	3000	MC	1	Evans
Asst C, CIS	O4	3000	MC	1	Boehm
Dietitian	O3	3420	AMS	1	Selle
Med Lab Tech	E7	92B30	MSC	2	Gaunt Hayes
Med Lab SP	E4	92B20	MSC	1	Dickinson
Sup Rsch Chem	I4	1320	GS	1	Bruton
Chem	I1	1320	GS	1	Smith
Sup Biol	I1	0401	GS	1	Davis
Sup Bio Tech	I0	0404	GS	1	Young
Med Tech	O9	0645	GS	2	Armstrong Burgess
Med Tech	O9	1320	GS	1	Dawson
	O9	0644	GS	1	Wright
	O9	0058	GS	1	Lukes
Biol Lab Tech	O8	0404	GS	2	Coleman Butler
Rsch Chem	O7	1320	GS	3	Maydonovitch Carmines Nelson
Med Tech	O7	0644	GS	2	Marcks Battista
Biol Lab Tech	O7	0404	GS	1	Barnes
Sec (Steno)	O6	0318	GS	1	Ervin
Clk (DMT)	O5	0316	GS	2	Laster Anastasi
Clk-Typist	O4	0322	GS	1	Keys
Clk (DMT)	O4	0316	GS	1	Glenn
Med Tech(Chem)	O4	0645	GS	1	Martin

Funding, FY-76:

Civilian Personnel	\$335,618
Mission Travel	12,000
Conference Travel	450
Rental	7,157
Contractual Services	9,036
Consumable Supplies	78,402
Nonexpendable Equipment	110,000

The Bailey K. Ashford Award for Clinical Research was established for presentation annually to the investigator whose research effort was judged to be the most significant contribution to the Clinical Investigation Program at WRAMC. The Award consists of a gold medallion and an appropriate certificate and presented at the graduation exercises of the residents and fellows. The recipient for 1975 was Major Timothy M. Boehm for his study entitled, "Synergistic Effects of Lithium and Iodine in the Treatment of Thyrotoxicosis".

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TABLE OF PUBLICATIONS AND PRESENTATIONS, FY-76

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in serum T3 and reverse T3, (Submitted for publication  
to J Clin Endocrinol & Metab, April 1976.

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- Adler RA, Noel GL, Wartofsky L, and Frantz AG: Failure of oral water loading and intravenous hypotonic saline to suppress plasma prolactin in man. *J Clin Endocrinol & Metab* 41:383-389, 1975.
- Burman, KD, et al: Klinefelter's syndrome: Examination of thyroid function and the TSH and PRL responses to TRH prior to and after testosterone administration. *J Clin Endocrinol & Metab* 41:1161-1166, 1975.
- Goldner FH, Boyce HW: The relationship of bile in the stomach to gastritis. *Gastrointestinal Endoscopy* 22: 197-199, May 1976.
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DEPARTMENT OF PEDIATRICS

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DEPARTMENT OF HEMATOLOGY, WRAIR

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Knodell RG, Conrad ME, Dienstag JL, Bell CJ: Etiological spectrum of posttransfusion hepatitis. Gastroenterol 69:1278-1285, 1975.

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DEPARTMENT OF HEMATOLOGY, WRAIR (continued)

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Intrinsic abnormalities of red cell metabolism of  
vitamin B6 in sideroblastic anemia. Presented at  
Third Meeting of the International Society of  
Haematology, European and African Div, London,  
England, August 1975, and published in Proceedings.

TITLE: Procainamide metabolism in renal failure and on hemodialysis

WORK UNIT NO: 1101

PRINCIPAL INVESTIGATOR: Thomas P. Gibson, MD, Major, MC

ASSOCIATE INVESTIGATOR: William A. Briggs, MD. LTC, MC and Edward Matusik,

OBJECTIVES: To determine the plasma half-life and renal excretion of procainamide in renal failure patients so that a method of dose reduction can be formulated to avoid excessive plasma levels.

TECHNICAL APPROACH: Subjects with normal renal function and patients on hemodialysis are given 500 mg of procainamide orally and blood for procainamide levels drawn at 15, 30, 45 and 60 minutes; every 30 minutes until 6 hours; hourly until 12 hours; and at 24 hours, if possible. All samples so collected are centrifuged and serum separated and frozen at -20°C until analysis.

Procainamide concentrations in serum and urine are determined by spectrophotofluorometry and spectrophotometry. The apparent half-life of procainamide in serum is determined from the slope of the terminal portion of a plot of log concentration versus time, by the method of least squares.

PROGRESS AND RESULTS: We describe a simple, rapid fluorometric assay for separate quantitative analysis of procainamide and N-acetylprocainamide in mixtures. The effective linear range (fluorescence vs. concentration) in serum is 0.1 to 10.0 mg/liter, regardless of the ratio (by weight) of the two drugs from 1:10 to 10:1. Analytical recoveries by the extraction method used were  $100.0 \pm 3.0\%$  and  $98.0 \pm 4.0\%$ , respectively. For determination of either compound, the maximum coefficient of variation was 10%.

Using the new method, serum concentrations of procainamide (PA) and N-acetylprocainamide (NAPA) were measured by fluorometry in subjects with normal renal function ( $n=4$ ) and in patients with end-stage renal failure ( $n=3$ ) after administration of 6.5 mg/kg of PA HCl orally. Two subjects with normal renal function were rapid isonicotinic acid hydrazide (INH) acetylators and two were slow acetylators. The rapid acetylators had higher peak serum levels of NAPA (1.80 ug/ml) than the slow acetylators (0.40 ug/ml). Peak serum levels of PA were essentially identical in both. The half-life ( $T_{1/2}$ ) of PA was shorter, 2.5 hr, in the rapid acetylators than in the slow, 4.1 hr. The slope of the terminal portion of the blood time curve for NAPA was steeper (-0.087) for slow acetylators than for rapid (-0.078). These apparent differences between rapid and slow acetylators

are not conclusive in themselves but tend to support the differences in acetylation previously reported. In the absence of renal function, the serum levels of PA were higher and the  $T_{\frac{1}{2}}$  prolonged. The serum levels of NAPA rose slowly and reached peak levels of 2 to 3 ug/ml and declined only with hemodialysis. In 3 patients measurable levels of NAPA were still present 78 hr (0.62 ug/ml), 94 hr (0.36 ug/ml), and 124 hr(0.70 ug/ml) after the single oral dose of PA. Clearance of NAPA during clinical hemodialysis was  $48 \pm 10$  cc/min compared to  $75 \pm 12$  ml/min for PA.

CONCLUSIONS: NAPA accumulates in renal failure patients.

FUNDS UTILIZED, FY-76: Laboratory Technician Salary, 20 hrs per week, \$5,500.00; Supplies \$800.00

FUNDING REQUIREMENTS, FY-77: None

PUBLICATIONS:

- 1) Matusik E, Gibson TP: Fluorometric assay for N-acetylprocainamide. Clin Chem 21:1899-1902, 1975.
- 2) Gibson TP, Matusik EJ, Briggs, WA: N-acetylprocainamide levels in patients with end-stage renal failure. Clin Pharmacol Ther 19:208-212, 1976.

TYPE OF REPORT: Terminated

TITLE: Resin hemoperfusion in the treatment of intoxications

WORK UNIT NO: 1103

PRINCIPAL INVESTIGATOR: William A. Briggs, MD LTC MC

ASSOCIATE INVESTIGATOR: Henry C. Yeager, MD Major MC

OBJECTIVES: To evaluate the effects of resin hemoperfusion in patients who have suffered drug intoxications.

TECHNICAL APPROACH: Patients who have become intoxicated with lipid-soluble drugs (such as Glutethimide) who are judged as critical and in whom benefit may result with the treatment by shortening of coma time. The patients will be treated by perfusion of their blood through a column containing Amberlite XAD-4 for a period of 2-4 hours with anticoagulation using Heparin. Vascular access will be used as in patients with Acute Renal Failure. Appropriate levels of the toxicant drug will be measured and will be used to guide further therapy.

PROGRESS AND RESULTS: None

CONCLUSIONS: None

FUNDS UTILIZED, FY-76: None

FUNDING REQUIREMENTS, FY-77: None

PUBLICATIONS: None

TYPE OF REPORT: Termination. The hemoperfusion cartridge has become generally available and is no longer a research item.

TITLE: Beta-adrenergic blockade in essential and renal hypertension

WORK UNIT NO: 1105

PRINCIPAL INVESTIGATOR: William A. Briggs, MD LTC MC

ASSOCIATE INVESTIGATOR: Thomas P. Gibson, MD Major MC

OBJECTIVES: To assess the efficacy and safety of propranolol as an antihypertensive agent and to determine whether its hypotensive effect is primarily dependent on suppression of plasma renin activity.

TECHNICAL APPROACH: Selected patients with moderate to severe hypertension are treated with propranolol in addition to other antihypertensive agents or hemodialysis. Blood pressure response is followed by standard clinical methods. Pharmacokinetics and dialysance studies have been done in some patients with chronic renal failure. Plasma renin activity has been determined prior to and after treatment and examined with respect to blood pressure response.

PROGRESS AND RESULTS: None

CONCLUSIONS: None

FUNDS UTILIZED FY-76: None

FUNDING REQUIREMENTS, FY-77: None

PUBLICATIONS: None

TYPE OF REPORT: Termination. No new patients have entered the study.

TITLE: Abnormalities of cellular immune mechanisms in acute renal failure

WORK UNIT NO: 1107

PRINCIPAL INVESTIGATOR: William A. Briggs, MD LTC MC

OBJECTIVES: To assess the frequency and severity of impaired cellular responsiveness to specific and nonspecific stimuli in vitro; to correlate such findings with infectious complications, and to assess the effect of dialysis on these parameters.

TECHNICAL APPROACH: Blood will be obtained from patients manifesting clinical evidence of acute renal insufficiency. Mononuclear cells will be isolated and cultured with plant mitogens and homologous cells, and the blastogenic response assessed by radiolabeled thymidine incorporation. Studies will be repeated after the patient has been treated with dialysis and after the start of the diuretic phase.

PROGRESS AND RESULTS: Following the establishment of a reliable and reproducible technique for culturing lymphocytes, lymphocyte response to three plant mitogens was assessed in patients receiving maintenance hemodialysis. Utilizing absolute counts per minute per culture, it was found that the vast majority of otherwise stable chronic dialysis patients had lymphocyte responsiveness in vitro similar to that seen with lymphocytes from control subjects. In addition, the response of patient lymphocytes to homologous antigens in one way mixed lymphocyte cultures was also generally noted to be equivalent to that seen with control lymphocytes. Thus, simply looking at thymidine incorporation did not bring out any abnormality of lymphocyte responsiveness universal to all patients. On the other hand, the lymphocyte response of some patients was clearly and consistently hyporesponsive. Additional studies were therefore undertaken to evaluate the modulation of plant mitogen response by cyclic nucleotides. A dose response relationship was established with molar concentrations of dibutyryl cyclic AMP ranging from  $10^{-2}$  to  $10^{-6}$ . Results were expressed as percent inhibition or stimulation utilizing the maximal PHA response without added cyclic nucleotide as the denominator. A difference in dose response relationship was seen in renal failure patients versus normal lymphocyte response. Lymphocytes from dialysis patients were inhibited in their PHA response in the presence of molar concentrations of dibutyryl cyclic AMP which had less or no inhibitory effect on the response of lymphocytes from control subjects. On the other hand, the enhancement of PHA responsiveness in the presence of cyclic GMP was similar utilizing either patient or normal lymphocytes.

CONCLUSIONS: By adding a modular of PHA responsiveness to the culture system of lymphocytes with plant mitogens it has been possible to develop an assay system which clearly separates the in vitro responsiveness of lymphocytes from patients with renal failure from that seen with lymphocytes from control subjects. As a result of the evolution and clearing of the abnormality in the effect of dialysis on lymphocyte dysfunction can be more accurately defined.

FUNDS UTILIZED, FY-76: Laboratory Technician salary, \$5,000. (six months)

PUBLICATIONS:

- 1) Webel ML, Briggs WA, Ritts RE: Studies of mitogen-induced lymphocyte transformation by a semi-microtechnique. Am J Clin Path 64:41-47, 1975.
- 2) Webel ML, Briggs WA, Ritts RE, Light JA: Lymphocyte blastogenesis in patients on chronic hemodialysis. Arch Int Med

TYPE OF REPORT: Terminated

TITLE: Acetylation of para-aminobenzoic acid (PABA) in normals and patients with reduced renal function (RRF).

WORK UNIT NO: 1108

PRINCIPAL INVESTIGATOR: Thomas P. Gibson, MD Major MC

ASSOCIATE INVESTIGATORS: Nesbitt D. Brown, William A. Briggs, LTC MC, Robert T. Lofberg PhD, and Gale Demaree, LTC, MSC

OBJECTIVE: To determine the effect of uremia and the anephric state on the ability of the liver to acetylate aromatic amines.

TECHNICAL APPROACH: Forty mgs of PABA in distilled H<sub>2</sub>O was given orally after an overnight fast. A urine and blood sample were taken prior to the solution. Thereafter blood and urine samples were taken every 15 minutes for the first hour, every 30 minutes for the next 2 doses, and hourly for the next 4 hours. All samples were centrifuged immediately and the separated serum frozen until analysis by high pressure liquid chromatography.

To date it has been found that after a single oral dose of PABA (40 mg/kg), 70% of the dose is recovered in the urine as p-aminohippuric acid (PAH) 10% as acetyl PAH, 10% as acetyl PABA, and 5% as PABA. Those individuals who are rapid acetylators of INH, appear to form large amounts of acetyl PAH and PABA and less PAH.

It has also been determined that the presently accepted methods of extraction of PAH from serum and urine underestimate the true amount of acetyl PAH and acetyl PABA and overestimate the PAH by virtue of hydrolysis of the acetyl compounds to their respective parent molecules. In addition, hydrolysis of PAH in 1N HCl at 100°C for 1 hour yields PABA and glycine, indicating the hydrolysis of amides leads to destruction of the molecule of interest.

PROGRESS AND RESULTS: None

CONCLUSIONS: Acetylation of PABA and PAH occurs. The extent is unknown and its relationship to INH acetylation phenotype needs to be further studied.

PUBLICATIONS:

- 1) Brown ND, Lofberg PT, Gibson TP: A study of the Bratton and Marshall hydrolysis procedure utilizing high performance liquid chromatography. Clinica Chem Acta, Accepted.

TYPE OF REPORT: Terminated

TITLE: Drug metabolism in chronic renal failure

WORK UNIT NO: 1110

PRINCIPAL INVESTIGATOR: William A. Briggs, MD LTC MC

ASSOCIATE INVESTIGATORS: David Lowenthal, MD Major MC,  
Thomas P. Gibson, MD Major MC

OBJECTIVES: To generate information on the pharmacokinetics of drugs which may be used in patients with renal insufficiency so that these drugs may be used in a safe and rational manner and minimize the risk of adverse drug reactions, especially in the more extremely ill or injured patient with acute renal insufficiency.

TECHNICAL APPROACH: Studies of drug metabolism are conducted in otherwise stable patients with chronic renal insufficiency because of potential difficulties and hazards in patients with acute renal failure. In general, drug concentrations are determined in serial blood (and sometimes saliva, urine or dialysate fluid) specimens after single oral, rarely parenteral, dosing of the patient. From the plotting of such data, it is possible to estimate absorption and elimination rate constants, volume of distribution and dialysance for the drug and to determine whether renal insufficiency modifies the metabolism and/or elimination of the drug.

PROGRESS AND RESULTS: Evaluation of the pharmacokinetics of acetaminophen including the impact of hemodialysis thereon was evaluated in patients with renal insufficiency in collaboration with Dr. Gerhard Levy, State University of New York at Buffalo. In these studies acetaminophen elimination by renal failure patients was not significantly different from normals; however, there was a pronounced accumulation of acetaminophen metabolites in these patients. Hemodialysis decreased the biologic half-life of acetaminophen by 42-53%. More importantly, hemodialysis served as the major route of elimination of the glucuronide and sulfate metabolites. In collaboration with Dr. Marcus Reidenberg, Cornell University School of Medicine, and evaluation of the plasma half-life of pentobarbital was undertaken in patients with renal insufficiency. These studies showed a substantial decrease in plasma half-life in some of the patients. This was considered most likely secondary to low apparent volumes of distribution rather than accelerated metabolism.

CONCLUSIONS: Patients with renal insufficiency often have low apparent volumes of distribution for parent compounds. Accumulation of metabolites represents a potentially significant problem in patients with renal insufficiency, especially when metabolites have significant biologic activity.

FUNDS UTILIZED, FY-76: None

FUNDING REQUIREMENTS, FY-77: None

PUBLICATIONS:

- 1) Oie S, Lowenthal DT, Briggs WA, Levy G: Effect of hemodialysis on pharmacokinetics of acetaminophen elimination by anephric patients. Clin Pharmacol Ther 18:680-686, 1975.
- 2) Lowenthal DT, Oie S, VanStone JC, Briggs WA, Levy G: Pharmacokinetics of acetaminophen elimination by anephric patients. J Pharmacol and Exper Ther
- 3) Reidenberg MM, Lowenthal DT, Briggs WA, Gasparo M: The plasma half-life of pentobarbital in patients with poor renal function.

TYPE OF REPORT: Terminated

TITLE: Use of Minoxidil in the treatment of severe, uncontrolled or poorly controlled hypertension.

WORK UNIT NO: 1112

PRINCIPAL INVESTIGATOR: William A. Briggs, MD LTC MC

ASSOCIATE INVESTIGATORS: Henry C. Yeager, MD Major MC; Thomas P. Gibson, MD Major MC

OBJECTIVES: Assess the efficacy and safety of minoxidil with severe hypertension refractory to available potent standard antihypertensive therapy.

TECHNICAL APPROACH: Hospitalized patients with diastolic hypertension in excess of 110 mm Hg and with organ damage from hypertension, despite treatment with a combination of a diuretic agent or ultrafiltration (dialysis) and hydralazine, aldomet or propranolol receive a trial of therapy with minoxidil. Effect on blood pressure and end organ damage and adverse reactions are noted and reported.

PROGRESS AND RESULTS: Since the establishment of this protocol, nine patients have been started on minoxidil for severe refractory hypertension. Three patients developed the refractory hypertensive state during the early post renal transplantation period on the basis of hyperreninemia secondary to renal rejection and/or ischemia as well as other factors which might have predisposed them to a hypertensive diathesis. Four patients had chronic renal failure being treated by maintenance hemodialysis when they developed severe refractory hypertension requiring the initiation of minoxidil therapy. One patient was started on minoxidil because of severe hypertension associated with renal infarction, presumably secondary to a form of polyarteritis. Another patient with presumed essential hypertension and progressive difficulty in control was started on minoxidil. In all instances, treatment with minoxidil was associated with an impressive decrease in mean arterial pressure and the ability to discontinue other antihypertensive agents. Propranolol has been utilized in association with the minoxidil to prevent reflex hemodynamic changes and in those patients with adequate renal function, diuretics have been utilized to prevent positive salt and water balance. Hypertrichosis has been the only clinically significant adverse affect from the minoxidil and one might anticipate this has been of most concern to our women.

CONCLUSION: Minoxidil is an extremely effective antihypertensive agent which has had a substantial positive impact on the ability to provide optimal blood pressure control in patient with otherwise severe refractory hypertension.

FUNDS UTILIZED, FY-76: None

FUNDING REQUIREMENTS, FY-77: None

PUBLICATIONS: None

TYPE OF REPORT: Interim

Principal Investigator is now Colonel D. E. Butkus, MC, Chief, Nephrology Service.

TITLE: Pharmacologic amelioration of glomerulonephritis with broad spectrum antihistaminic drugs.

WORK UNIT NO: 1114

PRINCIPAL INVESTIGATOR: William A. Briggs, MD LTC MC

ASSOCIATE INVESTIGATORS: Henry C. Yeager, MD Major MC, Thomas P. Gibson, MD Major MC

OBJECTIVE: To determine whether glomerular immune complex deposition and injury can be ameliorated by the use of drugs which antagonize vasoactive amines.

TECHNICAL APPROACH: Patients with idiopathic glomerulonephritis in which immune complex depositon and injury can be implicated by renal biopsy receive broad spectrum antihistamine or placebo in double blind fashion and the clinical course of the renal disease followed in standard fashion. This is a collaborative study with many other centers under the supervision of Dr. William T. Knicker, Professor of Pediatrics and Microbiology, University of Texas.

PROGRESS AND RESULTS: No patients have been entered into this study because the NIH Grant submitted by Dr. Knicker was never funded.

CONCLUSIONS: None

FUNDS UTILIZED, FY-76: None

FUNDING REQUIREMENTS, FY-77: None

PUBLICATIONS: None

TYPE OF REPORT: Termination. The study was never activated by Dr. Knicker because of lack of funding.

TITLE: Hyperalimentation and bilateral nephrectomy

WORK UNIT NO: 1115

PRINCIPAL INVESTIGATOR: William A. Briggs, MD LTC MC

ASSOCIATE INVESTIGATORS: Henry C. Yeager, MD Major MC; Mitchell V. Kaminski, MD Major MC; Jimmy A. Light, MD LTC MC

OBJECTIVES: To evaluate the effect of total parenteral nutrition with essential amino acids and hypertonic dextrose on nitrogen balance and clinical course of uremic patients following bilateral nephrectomy.

PROGRESS AND RESULTS: No new patients have entered the study.

CONCLUSIONS: None

FUNDS UTILIZED, FY-76: None

FUNDING REQUIREMENTS, FY-77: None

PUBLICATIONS:

Kaminski, MV, et al: Hyperalimentation and bilateral nephrectomy. Acta Scan Chirurgica (In press). Presented at the Tenth International Congress, Kyoto, Japan, June 1975.

TYPE OF REPORT: Termination. No new patients have entered the study.

TITLE: Studies on the Metabolic Fate and Toxicity of Di-2Ethylhexyl Phthalate (DEHP) Infused to Patients During Hemodialysis.

WORK UNIT NO: 1116

PRINCIPAL INVESTIGATOR: Thomas P. Gibson, MD Major MC

ASSOCIATE INVESTIGATORS: William A. Briggs, M.D. LTC MC and Betty J. Boone, Ph.D

OBJECTIVES: To determine the quantity of di-2-ethylhexyl phthalate (DEHP) delivered to patients during routine clinical hemodialysis.

TECHNICAL APPROACH: Patients requiring maintenance renal hemodialysis were studied. Hemodialyses were performed using recirculating single-pass machines and coils. Blood samples for DEHP analysis were collected in glass syringes with metal needles. A predialysis venous sample was obtained before the patient was connected to the dialysis tubing. During hemodialysis, blood was drawn from the venous and arterial lines at sites 20 cm from the patient; venous being withdrawn after arterial samples. After onset of dialysis samples for DEHP analyses were obtained at 15 and 30 minutes, 1, 2, 3, 4, and 5 hours, and immediately after dialysis unless dialysis was terminated earlier. All samples were placed into acid-washed glass test tubes, centrifuged, the plasma separated and placed into glass vials. Samples were frozen at -80°C. until analyzed by gas liquid chromatography.

PROGRESS AND RESULTS: The amount delivered increased with the length of dialysis, up to 150 mg during a 5 hour hemodialysis. The metabolic fate and toxicity, if any, of infused DEHP remains to be determined.

CONCLUSIONS: The amount delivered increased with the length of dialysis, up to 150 mg during a 5 hour hemodialysis. The metabolic fate and toxicity, if any, of infused DEHP remains to be determined.

FUNDS UTILIZED, FY-76: None

FUNDING REQUIREMENTS, FY-77: None

PUBLICATIONS:

- 1) Gibson TP, Briggs WA, Boone BJ: Delivery of di-2-ethylhexyl phthalate to patients during hemodialysis. J Lab Clin Med 87:519-524, 1976.

TYPE OF REPORT: Termination. The data presented above constitutes all that has been completed to this time. The project was terminated because of the RIF at WRAIR and because another project of higher priority removed Dr. Boone from this study.

TITLE: Pharmacokinetics of propoxyphene (Darvon) in anephric patients and in normal subjects.

WORK UNIT NO: 1117

PRINCIPAL INVESTIGATOR: Thomas P. Gibson, MD Major MC

ASSOCIATE INVESTIGATOR: William A. Briggs, MD LTC MC

OBJECTIVE: To determine if renal failure reduces the first pass effect metabolism of propoxyphene.

TECHNICAL APPROACH: Subjects will be fasted overnight and for 4 hours after drug administration. A single dose of 130 mg d-propoxyphene hydrochloride (2 Darvon 65 mg capsules, dissolved in 20 ml water, with 40 ml Coca Cola Syrup then added) will be administered orally at 0800 hours. The container will be rinsed with two 15 ml portions of water which will also be ingested. Food will be withheld for 4 hours after dosing. Ten ml venous blood will be obtained in vacutainers containing EDTA as anticoagulant at ~5 minutes, and at 0.5, 1, 2, 3, 5, 7, 9, 12, 16 where possible, and 24 hours after drug administration. In the normal subjects, blood samples will also be obtained at 30, 36, and 42 hours. Plasma will be separated at once, quick-frozen, and stored at -20°C pending assay. Mixed saliva will be obtained at ~5 minutes, and at 1, 3, 5 and 7 hours. The mouth will be rinsed twice with water, a square of Parafilm rolled into a ball will be chewed, and saliva will be expectorated directly into a small vial for 3 minutes or until at least 3 ml of saliva has been collected. These samples will also be frozen. Some of the patients will also be studied during hemodialysis. Drug will be administered immediately after the start of dialysis and blood samples will be obtained from the arterial and venous sides of the artificial kidney.

The following laboratory studies will be obtained on the ~5 minute blood sample; total protein, albumin, BUN, creatinine, and hematocrit. During hemodialysis BUN will be measured on all samples by Dr. Gibson of the Dept of Nephrology. Renal function in normals and patients will be estimated by the 24 hours clearance of endogenous creatinine.

PROGRESS AND RESULTS: To date eight patients requiring maintenance hemodialysis have been studied. The results indicated that the apparent half life of darvon is not statistically different when compared to published results from normal subjects. The levels of norpropoxyphene do appear to be higher in the renal patients. The salivary findings indicated that it may be possible to monitor blood levels of the parent and metabolite by measuring the concentration in saliva. The sketchy results presented here are due to the fact that the complete evaluation is not complete at this time.

CONCLUSIONS: The metabolites of darvon does not appear to be significantly altered by renal disease. However, the metabolite norporpoxyphene does appear to accumulate.

FUNDS UTILIZED, FY-76: None

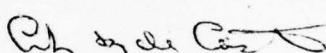
FUNDING REQUIREMENTS, FY-77: None

PUBLICATIONS: None

TYPE OF REPORT: Terminated

# DISPOSITION FORM

For use of this form, see AR 340-15; the proponent agency is The Adjutant General's Office.

REFERENCE OR OFFICE SYMBOL	SUBJECT
HSW-MC	Annual Progress Report, Clinical Investigation Program
TO THRU Ch, Dept of Med /	FROM Ch, Cardiology Svc
	DATE 8 June 1976 CMT 1
TO Ch, Clinical Invest Svc	
1. Projects #1209 and #1210 regarding The Natural History of Ventricular Septal Defects and The The Registry for Collection of Data from Total Army Experience in Coronary Artery Surgery have had no progress during the past 12 months.	
2. I suggest these projects be dropped from your active list until the Cardiology Service can rehire a research registry clerk who can devote full time to these and other projects.	
 CARLOS M. de CASTRO COLONEL, MC CHIEF, CARDIOLOGY SERVICE	

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DA FORM 1 FEB 62 2496

REPLACES DD FORM 36, EXISTING SUPPLIES OF WHICH WILL BE ISSUED AND USED UNTIL 1 FEB 63 UNLESS SOONER EXHAUSTED.

★ EPO 1971 - 441-650

Work Unit No.: 1304

Title of Project: Diurnal Variation in Prolactin Release in Humans

Investigators:

Principal: Gordon L. Noel, MAJ, MC

Associates: L. Wartofsky, LTC, MC; J.M. Earll, COL, MC;  
A.G. Frantz, MD

Objectives: To study the secretory patterns of prolactin in humans with thyroid and pituitary disease, breast cancer, and galactorrhea.

Technical Approach: Plasma for prolactin (2 ml) is obtained at one hour intervals from patients throughout a 24 hour period. Prolactin is measured with a specific human prolactin immunoassay by Dr. A.G. Frantz, Columbia University College of Physicians and Surgeons, New York, N.Y.

Progress and Results: Studies of patients with idiopathic galactorrhea reveal normal 24 hour prolactin secretory patterns eliminating nocturnal prolactin hypersecretion as the cause of this form of galactorrhea.

Conclusions: The technique of diurnal sampling of prolactin is successful. L-dopa does not provide consistent and effective suppression of the nocturnal release of prolactin. Further studies are planned when the more effective agent, Lilly Compound 83636, becomes available.

Publication: None

Type of Report: Terminated - reason for termination is principal investigator has departed and the most potentially productive portion of this protocol will be studies with the Lilly compound 83636 which will be carried on work unit number 1320. In addition, more sophisticated multisample sleep studies have now been published by other investigators.

Work Unit No.: 1306

Title of Project: Comparison of Conventional Irradiation with Transsphenoidal Surgery Under Direct Vision in Treatment of Acromegaly

Investigators:

Principal: Jerry M. Earll, COL, MC

Associates: Marcus Schaaf, M.D.; Albert N. Martins, LTC, MC and Richard C. Dimond, MAJ, MC

Objectives: To evaluate the effectiveness of a new transsphenoidal microsurgical approach to the pituitary in treating acromegaly.

Technical Approach: A dissecting microscope and a transsphenoidal approach to the sella turcica permits operation on the pituitary under direct microscopic vision. Acromegalic patients and later, others with pituitary adenomas will be operated upon and the results compared with conventional therapy like radiation.

Progress and Results: Serum growth hormone levels, thyroid function, and adrenal function were measured before and after surgery in 16 of 17 acromegalic patients undergoing transnasal transsphenoidal microsurgery of the pituitary. Thirteen patients have been followed up for 12 to 24 months; three patients have been followed up for three to six months. Serum growth hormone levels decreased to less than 5 ng/ml in seven of nine previously untreated patients; thyroid and adrenal function were preserved in eight of these nine patients. In seven patients treated previously by other modes of therapy, growth hormone levels after transsphenoidal surgery decreased to less than 5 ng/ml in three, to between 5 and 10 ng/ml in three, and from 98 to 41 ng/ml in one. Preoperative adrenal function was normal in six of these seven patients and was preserved in four; thyroid function was normal in five patients preoperatively and was preserved in three.

Conclusions: Transsphenoidal micorsurgery appears to offer an effective means of lowering growth hormone levels and a possibility of preserving any remaining normal pituitary function. It may be considered for initial treatment in selected patients in whom more rapid arrest of acromegaly is indicated.

Publications: Atkinson, R.L., D.P. Becker, A.N. Martins, M. Schaaf,  
R.C. Dimond, L. Wartofsky and J.M. Earll. J.A.M.A.  
233: 1279-1283, 1975.

Acromegaly: Treatment by Transsphenoidal Microsurgery.

Type of Report: Completed.

Long term follow-up on these patients will be accomplished  
and may be a subject of a much later report, but there  
is no reason for an annual progress report on this  
particular protocol.

Work Unit No.: 1308

Title of Project: Inderal Kinetics in Hyperthyroidism

Investigators:

Principal: Ken Burman, MAJ MC

Associates: L. Wartofsky, LTC, MC; Dave Lowenthal, M.D.  
William Briggs, LTC, MC, Jerry Earll, COL, MC

Objectives: To assess Inderal half life in thyrotoxic patients.

Technical Approach: All patients are studied on Ward 30. They are given Inderal and blood samples are obtained to ascertain its disappearance rate.

Progress and Results: About 15 patients have been analyzed and preliminary data indicates that the half life of Inderal may be about 90 minutes which is not different than a group of normal controls.

Conclusions: Inderal half life is about one hour.

Funds Utilized FY 76: None

Funding Requested FY 77: None

Publications: None

Type of Report: Interim (Annual)

Work Unit No.: 1310

Title of Project: TRH in Patients with Hypothalamic Pituitary Thyroid Disease

Investigators:

Principal: Leonard Wartofsky, LTC, MC

Associates: R.C. Dimond, MAJ, MC, M. Schaaf, M.D.; J.M. Earll, COL, MC; G.L. Noel, MAJ, MC

Objectives: To assess the response to synthetic TRH (Thyrotropin releasing hormone) in various suspected endocrine disorders.

Technical Approach: Patients are studied on the metabolic ward. Blood samples are drawn for measurement of thyrotropin prolactin, and other hormones, before and after the injection of 100-500 mcg of synthetic TRH. The latter agent is currently an investigational drug distributed by Abbott Laboratories for Phase 3 clinical testing.

Progress and Results: Approximately 400 such studies have been completed in approximately 250 subjects. Although much of the data is yet to be analyzed, some appears in the publications listed below.

Conclusions: TRH has been found to be a useful agent for the assessment of disorders of the hypothalamic-pituitary-thyroid axis, with minimal or negligible side effects or problems associated with its use; and has also proved to be a valuable research tool.

Funding Requested FY-77: None

- Publications:
1. Noel, G., R.C. Dimond, L. Wartofsky, J.M. Earll and A.G. Frantz. Continuous Infusion of TRH in Man. J. Clin. Endocrinol. 38:6-17, 1974.
  2. Wartofsky, L., R.C. Dimond, G.L. Noel, R.A. Adler, A.G. Frantz, and J.M. Earll. Effect of Water Loading on TSH and PRL Responses to TRH. J. Clin. Endocrinol. & Metab., 41:784-787, 1975.

Work Unit No. 1310 (cont)

3. Wartofsky, L., et al., Failure of Propranolol to alter TSH and PRL Responses to TRH in Thyrotoxicosis, J. Clin Endocrinol Metab, 41:484-490, 1975.
4. Wartofsky, L., et al., Estimates of Pituitary Stores of TSH and PRL in Normal and Hypothyroid Subjects by Use of Continuous TRH Infusion, Advances in Thyroid Research, Excerpta Medica, pp. 268-271, 1976.
5. Wartofsky, L., et al., Effect of Acute Increases in Serum T3 on TSH and PRL Responses to TRH, J. Clin. Endocrinol & Metab., 42:451-466, 1976.

Type of Report: Interim (Annual)

Work Unit No.: 1311

Title of Project: Treatment of Thyroid Storm with Anion Exchange Resin.

Investigators:

Principal: Kenneth D. Burman, MAJ, MC

Associates: H. Yeager, MAJ, MC; J.M. Earll, COL, MC; W. Briggs;  
L. Wartofsky, LTC, MC

Objectives: To treat thyroid storm with an anion exchange resin.

Technical Approach: Patients will undergo extracorporeal hemoperfusion.

Progress & Results: No patients studied to date because no patient ill enough to fit criteria and require this therapy.

Conclusions: None

Funding Utilized FY-76: None

Funding Requested FY-77: None

- Publications:
1. Burman, K.D., et al. Resin Hemoperfusion: A Method of Removing Circulating Thyroid Hormones. J. Clin. Endocrinol & Metab, 42:70-78, 1976.
  2. Burman, K.D., et al., Resin Hemoperfusion: A Potential New Treatment for Thyroid Storm. Proceedings 6th International Thyroid Congress, Advances in Thyroid Research, Excerpta Medica, pp. 217-220, 1976.

Type of Report: Interim (Annual)

Work Unit No: 1312

Title of Project: TRH Testing During Acute Malaria

Investigators:

Principal: Leonard Wartofsky, LTC, MC

Associates: R.C. Dimond, MAJ, MC; J.M. Earll, COL, MC

Objectives: To investigate the release of TSH and prolactin (HPr) from the pituitary during acute malaria.

Technical Approach: TSH and HPr were measured before and during acute malaria after stimulation with TRH (TSH-releasing hormone). Previous studies had suggested pituitary suppression during this infection, and these studies were designed to ascertain whether the level of suppression was at the hypothalamus or the pituitary.

Progress and Results: Studies have been completed in four volunteers. The data indicates that TSH responses to TRH are intact but PRL responses are slightly increased. Serum reverse T3 levels were also found to vary reciprocally with T3 with decreases in T3 and increases in rT3 during peak infection.

Conclusions: The nature of thyroidal suppression during malaria appears to involve effects at both the periphery and at a hypothalamic level.

Funding Utilized FY-76: None

Funding Requested FY-77: None

Publications: Wartofsky, L., et al., Studies on the Nature of Thyroidal Suppression During Acute Falciparum Malaria: Integrity of Pituitary Response to TRH and Alterations in Serum T3 and Reverse T3, submitted for publication to J. Clin. Endocrinol. & Metab., April 1976.

Type of Report: Completed

Work Unit No.: 1313

Title of Project: Evaluation of Growth Hormone Secretory Responses  
in Acromegaly

Investigators:

Principal: LTC Richard C. Dimond

Associates: R.L. Atkinson, Jr., MAJ K. Burman, COL J.M. Earll

Objectives: To determine if variability in patterns of growth hormone secretion is characteristic of acromegaly.

Technical Approach: Periodic standard oral glucose, insulin, and arginine tolerance tests to evaluate growth hormone secretory responses.

Progress and Results: This project was initiated in 1972 based upon preliminary observations in two small groups of patients with acromegaly undergoing drug therapy with Provera (6 patients) or Thorazine (8 patients). Subsequently, the commercially available preparation of arginine was withdrawn from the market and assignment of research priorities precluded the routine performance of insulin tolerance tests in our acromegalic patients. Since most of the patients were unable to be tested serially with all three provocative agents, the continuation of this protocol as designed has not been possible. Therefore, this project cannot be completed and is terminated at this time without being able to draw any conclusions. Copies of the published preliminary data are attached.

Conclusions: None

Funds Utilized FY-76: None

Funding Requested FY-77: None

Publications (FY-76: None

Type of Report: Terminated.

Work Unit No.: 1314

Title of Project: Stimulation and Suppression of Plasma Prolactin in Patients with Pituitary Disease

Investigators:

Principal: Gordon L. Noel, MAJ, MC

Associates: J.M. Earll, COL, MC; A.G. Frantz, MD

Objectives: 1. To evaluate the efficacy of various tests now available in stimulating and suppressing the release of prolactin as a means of assessing pituitary function.

2. To study the pathophysiology of abnormal prolactin secretion in various forms of galactorrhea.

3. To ascertain whether prolactin has osmoregulatory properties in patients with disorders of prolactin secretion.

Technical Approach: Patients with pituitary tumors and/or galactorrhea have complete evaluation of their pituitary function by standard methods (thyroid and adrenal function studies, growth hormone stimulation and suppression). Following this, tests of prolactin release (TRH test, chlorpromazine stimulation, breast stimulation, sleep and of prolactin suppression (L-dopa, water loading) are performed. Prolactin is measured in an immunoassay by Dr. A.G. Frantz, Columbia University College of Physicians and Surgeons, New York, N.Y.

Progress and Results: Study of 29 patients with galactorrhea and/or pituitary tumors reveal that basal prolactin determinations correlated better with the presence of a pituitary tumor than all of the stimulatory and suppressive manipulations. A small but statistically significant rise in mean prolactin was found in 10 normal men and 11 normal women one half hour after ingestion of a water load. There was no effect of intravenous infusion of either hypotonic or hypertonic saline.

Conclusions: Basal prolactin concentrations are more useful than other methods of differentiating between tumorous and non-tumorous galactorrhea. TRH and chlorpromazine testing are effective means of establishing the integrity of the pituitary and

hypothalamic pituitary interactions. Water loading studies did not support a physiologic role for prolactin in the short term regulation of plasma osmolality in humans.

Funding Requirements: N/A

Publications: Adler, R.A., G.L. Noel, L. Wartofsky, and A.G. Frantz. Failure of Oral Water Loading and Intravenous Hypotonic Saline to Suppress Plasma Prolactin in Man. J. Clin. Endocrinol. & Metab. 41:383-389, 1975.

Type of Report: Interim (annual)

Work Unit No.: 1320

Title of Project: Effect of Lilly Compound 83636 on Plasma Prolactin in Humans with Galactorrhea, Probably Pituitary Tumors, or Breast Cancer

Investigators:

Principal: Jerry M. Earll, COL, MC

Associates: M. Schaaf, M.D., T. Boehm, MAJ, MC, G. Jones, MAJ, MC

Objectives: (1) To study the physiology of prolactin release in patients with probable pituitary tumors and breast carcinoma. (2) To study the effect of Cmpd 83636 on known stimuli to prolactin release. (3) To study the efficacy of Cmpd 83636 as therapy for galactorrhea (4) To study the efficacy of Cmpd 83636 in lowering prolactin concentrations in patients with metastatic breast carcinoma.

Technical Approach: Samples of plasma prolactin are to be obtained during sleep, and after thyrotropin-releasing-hormone or phenothiazine-stimulated prolactin release, both before and during short term treatment with Cmpd 83636 to estimate the efficacy of Cmpd 83636 in blocking both natural and pharmacologic stimuli to prolactin secretion. Cmpd 83636 is to be given in courses of up to 90 days to study its efficacy in the treatment of galactorrhea and as a prolactin-lowering agent in patients with metastatic carcinoma of the breast.

Progress and Results: Receipt of the drug has been delayed during the course of the review of the data presently available to the FDA. The drug is just now being released and this study will begin in the near future.

Conclusions: None yet.

Funding Requirements: Funding will be handled by general contract to Kyle Metabolic Unit.

Publications: None

Type of Report: Interim

Work Unit Number: 1321

Title of Project: Effects of Cancer Chemotherapy Agents on Endocrine Function

Investigators:

Principal: COL Jerry M. Earll, MC

Associates: MAJ John H. MacIndoe, MC

Objectives: To evaluate the effects of modern cancer chemotherapy on endocrine function.

Technical Approach: Patients receiving standard protocol approved drugs have their endocrine function tested before and after treatment. No change is made in the patients usual management for malignancy.

Progress and Results: Five patients have had evaluation of their adrenal function following completion of either one or two courses of chemotherapy. Two other patients had been entered in the study but became ill with hepatitis. No significant suppression of adrenal function was detected following the early courses of chemotherapy. A pronounced hyperzincuria occurred within 24 hours of the administration of most chemotherapeutic agents while serum zinc levels remained stable.

Conclusions: Preliminary results suggest minimal if any significant impairment of adrenal function following initial courses of chemotherapy. The immediate hyperzincuria following certain chemotherapeutic agents may reflect drug toxicity upon tumor and normal tissue cells at a time much sooner than traditional concepts would suggest. Gonadal and thyroid function changes will now be pursued as described in the protocol.

Funding Requirements: None

Publications: None

Type of Report: Interim (Annual)

Work Unit No.: 1329

Title of Project: Lithium Effects on Thyroid Gland

Investigators:

Principal: Kenneth D. Burman, MAJ MC

Associates: L. Wartofsky, LTC MC, R.C. Dimond, MAJ MC, J.M. Earll,  
COL MC

Objectives: To determine the effects of lithium upon the thyroid gland  
and upon thyroid function in patients with euthyroid Graves'  
Disease.

Technical Approach: To measure T<sub>3</sub>, T<sub>4</sub> and TSH in patients with  
Euthyroid Graves' Disease given lithium for  
approximately 6 weeks.

Progress and Results: 7 Patients have been studied and preliminary  
results indicate lithium will lower T<sub>3</sub> and T<sub>4</sub>  
levels to a degree suggesting these patients  
have enhanced sensitivity.

Conclusions: Patients with euthyroid Graves' Disease may be particularly  
susceptible to lithium-induced hypothyroidism.

Funds Utilized FY 76: \$250.00 Equipment  
77.73 Travel & TDY  
\$327.73

Funding Requested FY77: None

Publications: None.

Paper presented at Annual Meeting of American College  
of Physicians, Philadelphia, Pa., April 1976.

Type of Report: Interim (Annual).

Work Unit No.: 1330

Title of Project: Thyroid function in patients with Klinefelter's Syndrome

Investigators:

Principal: Kenneth D. Burman, MAJ MC

Associates: L. Wartofsky, LTC, MC, G.L. Noel, M.D., R.C. Dimond,  
LTC, MC, J.M. Earll, COL, MC

Objectives: To determine if thyroid function is normal in patients  
with Klinefelter's Syndrome

Technical Approach: Serum T<sub>3</sub>, T<sub>4</sub>, TSH, RAIU, and the TSH and PRL  
responses to TRH both prior to and during  
testosterone therapy were assessed.

Progress and Results: 7 patients have been studied and results indicate  
that T<sub>3</sub>, T<sub>4</sub> and TSH levels are normal in men  
with Klinefelter's Syndrome. Prolactin responses  
to TRH were augmented over normal.

Conclusion: Patients with Klinefelter's Syndrome appear to have  
normal thyroid function, but abnormal PRL responses to  
TRH.

Funds Utilized FY-76: Equipment \$250.00

Funds Requested FY-77: None

Publications: Burman, K.D. et al. Klinefelter's Syndrome: Examination  
of Thyroid Function and the TSH and PRL responses to  
TRH prior to and after Testosterone Administration. J.  
Clin. Endocrinol. & Metab. 41:1161-1166, 1975.

Type of Report: Interim (Annual).

Work Unit Number: 1331

Title of Project: Effect of Iodine and Lithium on the Release of Thyroxine from the Thyroid Gland of Patients with Thyrotoxicosis

Investigators:

Principal: Timothy M. Boehm, MAJ MC

Associates: K.D. Burman, MAJ MC, L. Wartofsky, LTC MC

Objective: Both lithium and iodine block thyroidal secretion and lead to clinical improvement in patients with thyrotoxicosis. This study is designed to examine whether a synergistic effect can be demonstrated by administration of both agents.

Technical Approach:  $^{125}\text{I}$  and  $\text{T}_4$   $^{131}\text{I}$  are given to label the thyroid and peripheral pools respectively. Blood is drawn bidaily and urines collected 12 hourly during 5 day control periods and during two 5 day treatment periods with either lithium or iodine followed by both drugs.

Progress and Results: 18 patients have completed study; lithium and iodine are comparably efficacious agents in blocking thyroidal release. Additional therapeutic benefit was observed if lithium was added to iodine therapy but not if iodine was added to lithium. This "conditional" synergism was observed regardless of whether methimazole was employed. Further studies are underway to ascertain whether this "synergism" is a cumulative benefit of iodine administration.

Conclusions: Lithium and iodine appear to be conditionally synergistic and may mechanistically affect different aspects of thyroidal release.

Funds Utilized, FY-76: None

Funding Requirements, FY-77: None

Publications: None

Type of Report: Interim

Work Unit No.: 1332

Title of Project: Differentiation of Benign from Malignant Thyroid  
Nodules: Assessment of New Diagnostic Techniques.

Investigators:

Principal: Robert Smallridge, MAJ MC

Associate: Leonard Wartofsky, LTC MC, Dominic Corrigan, LTC, MC,  
Prentice Thompson, MAJ, MC Robert Corcoran, MAJ, MC

Objectives: To attempt to differentiate benign from malignant thyroid  
nodules by use of the routine I<sup>131</sup> and Technetium 99<sup>m</sup> scans  
plus newer diagnostic techniques including ultrasonography,  
fluorescent scanning and needle biopsy.

Technical Approach: 50-75 patients with solitary thyroid nodules will  
have the diagnostic tests mentioned under objectives  
which will be correlated with findings at surgery  
in hope of preventing the number of patients requiring the latter.

Progress and Results: 35 patients have been included in the study thus  
far with only 18 having gone to surgery. Thus,  
there are too few patients to draw meaningful  
conclusions.

Conclusions: None yet.

Funding Utilized FY-76: Equipment \$250.00

Funding Requested FY-77: None

Publications: None

Type of Report: Interim (Annual).

Work Unit No.: 1333

Title of Project: Effects of Clofibrate and Chlorpropamide in Vasopressin-sensitive Diabetes Insipidus.

Investigators:

Principal: P. Thompson, MAJ MC

Associates: J. Earll, COL MC and M. Schaaf, M.D.

Objectives: To compare the effectiveness of clofibrate and chlorpropamide both singly and in combination in patients with diabetes insipidus.

Technical Approach: 8 patients with vasopressin-sensitive diabetes insipidus underwent water deprivation and water load tests under the following conditions:

1. No therapy
2. On clofibrate or chlorpropamide (the selection of which was randomized and then reversed).
3. On both clofibrate and chlorpropamide in combination

Progress and Results: Six patients with vasopressin-responsive diabetes insipidus received cloribrate and chlorpropamide, singly and in combination. Decrease in urinary output averaged (mean  $\pm$  SE): Clofibrate, 2 gm/d,  $44 \pm 7\%$ ; chlorpropamide, 250 mg/d,  $50 \pm 8\%$ ; clofibrate, 2 gm/d and chlorpropamide, 125 mg/d,  $48 \pm 6\%$ ; clofibrate, 2 gm/d and chlorpropamide, 25- mg/d,  $59 \pm 3\%$ . Water deprivation tests before and during treatment showed significantly higher basal, final and peak urinary osmolalities ( $U_{osm}$ ) and lower free water clearance ( $C_{H2O}$ ) on chlorpropamide, singly and in combination; clofibrate raised  $U_{osm}$  less but significantly decreased  $C_{H2O}$ . Water load tests before and during treatment showed that chlorpropamide, singly and in combination, markedly decreased maximal urinary flow, maximal  $C_{H2O}$ , % water load excreted, and increased minimal  $U_{osm}$ ; clofibrate significantly decreased maximal urinary flow and  $C_{H2O}$  only. One patient responded only to combination therapy. Chlorpropamide caused

serious hypoglycemia in 3 of 6 patients.  
Clofibrate had no significant side effects.

Conclusions: Oral agent are effective with patients with partial diabetes insipidus. In selecting an oral agent for treating diabetes insipidus we advise initial trial of clofibrate; if ineffective, low dose chlorpropamide may be tried with precautions to avoid hypoglycemia. An occasional patient who is unresponsive to chlorpropamide and clofibrate alone may respond to combination therapy.

Funding Requirements: N/A

Publications: Manuscript submitted Archives of Internal Medicine.

Type of Report: Completed

Work Unit No.: 1334

Title of Project: The Regulation of Extrathyroidal Conversion of Thyroxine (T4) to Triiodothyronine (T3)

Investigators: Principal: Kenneth D. Burman, MAJ MC

Associate: L. Wartofsky, LTC MC

Objectives: It is generally accepted that 80-90% of circulating T<sub>3</sub> in man is derived from the monodeiodination of T<sub>4</sub>. We plan to examine whether the proportion of T<sub>4</sub> converted to T<sub>3</sub> will vary depending upon the existing serum concentrations of T<sub>4</sub> and T<sub>3</sub>.

Technical Approach: Patients who have been thyroidectomized for thyroid cancer and who are on fixed replacement doses of T<sub>4</sub> will be studied. Conversion of T<sub>4</sub> to T<sub>3</sub> is calculated from the kinetics of disappearance of injected doses of <sup>131</sup>I-T<sub>4</sub> and <sup>125</sup>I-T<sub>3</sub>. Conversion will be quantitated and compared while patients are receiving varied doses of T<sub>4</sub> and/or T<sub>3</sub> replacement.

Progress and Results: 9 patients have been studied. The results indicate that the percentage of T<sub>4</sub> and T<sub>3</sub> conversion remains constant regardless of the dose of T<sub>4</sub> given.

Conclusions: T<sub>4</sub> to T<sub>3</sub> conversion varies independently with the serum T<sub>4</sub> level.

Funds Utilized FY 76: Equipment \$500.00

Funds Requested FY-77: None

Publications: None  
Paper presented at National Meeting of American Federation of Clinical Research, Atlantic City, N.J., May 2, 1976.

Type of Report: Interim (annual)

Work Unit No.: 1335

Title of Project: Intestinal Bile Salt Clearance in Thyrotoxic Patients  
With and Without Diarrhea

Investigators:

Principal Investigator: Maj Mark Donowitz, MC

Associate Investigators: Maj Dean Kinsey, MC; LTC Leonard Wartofsky  
MC; and Maj Kenneth Burman, MC, Maj Tim Boehm,  
MC

Objectives: To determine nature and quantity of bile salts in serum,  
jejunum both fasting and post-prandially and in stool in  
patients with and without diarrhea and hyperthyroidism.

Technical Approach: Serum was obtained fasting; stool is collected and  
frozen over a 3 day period; bile is collected by  
passing a single lumen tube overnight and in the  
morning prior to ingestion of food, fasting jejunal  
contents and jejunal contents following Freamine  
injection are obtained. These samples are extracted  
and studied quantitatively for bile salts by gas  
liquid chromatography.

Progress and Results: (See accompanying abstract published in Gastroenterology  
70: 902, 1976 and presented at annual meeting of  
American Gastroenterology Association, Miami, Florida,  
May 28, 1976.)

A sensitive and specific radioimmunoassay for conjugates of  
chenodeoxycholic acid was developed. Antiserum was  
developed in rabbits injected at weekly intervals for 8  
weeks with glycochenodeoxycholic acid conjugated to Bovine  
Serum Albumin by the carbodiimide method. The displacement  
curve over the range of 4 to 80 pmoles was linear when  
the percent binding was plotted against the natural log  
of the added concentration utilizing a 1:250 dilution of  
antibody.

The specificity of the RIA is illustrated by the relative  
amounts of pure conjugated bile acid standards required  
to displace 50% of bound  $^3\text{H}$ -glycochenodeoxycholic acid with

glychenodeoxycholate as reference. The antibody has 400 times less affinity for glycocholate and 3000 times less affinity for glycodeoxycholate. There was equal reactivity between glycine and taurine conjugates and only 1 log difference with free chenodeoxycholic acid. The assay is thus capable of sensitive and specific measurements of conjugates of chenodeoxycholic acid in serum.

Eight patients with hyperthyroidism with pruritus and 5 without pruritus have been studied and compared to normal volunteers. Patients with hyperthyroidism have a shift in their primary biliary bile acid from cholic acid to chenodeoxycholic acid. Patients with pruritus and hyperthyroidism have elevated serum dhenodeoxy acid levels compared to controls which exceed that of normals. Hyperthyroid patients without pruritus have normal serum chenodeoxycholic acid levels. Following conventional treatment for hyperthyroidism with propylthiouracil, pruritus stops and serum chenodeoxycholic acid levels return towards normal.

- Conclusions:
- a) Serum chenodeoxycholic levels can be measured by a sensitive specific radioimmunoassay.
  - b) Patients with hyperthyroidism have an increase in biliary chenodeoxycholic acid.
  - c) Pruritus is a common symptom in hyperthyroidism.
  - d) Serum chenodeoxycholic levels are increased in hyperthyroid patients with pruritus.
  - e) Medical treatment of hyperthyroidism is associated with resolution of pruritus and return of serum chenodeoxycholic levels towards normal.

Funding Requirements:

Personnel: Hospital, none outside of nurses on Ward 30

Equipment: All supplied by Investigator's laboratories at WRAIR.

Funding Requested: \$500.00 for TDY

Publication: Gastroenterology 70: 902, 1976

Type of Report: Interim

Work Unit No: 1336

Title of Project: Effects of Continuous Infusion of TRH on Growth Hormone Secretion in Acromegaly.

Investigators:

Principal: LTC Richard C. Dimond, MC

AssociateS: LTC D. Corrigan, LTC L. Wartofsky

Objectives: To examine the pattern of growth secretion in patients with acromegaly during continuous infusion of TRH.

Technical Approach: TRH is administered intravenously by constant infusion at a rate of 1 microgram/min for 4 hours and then by a 500 microgram bolus injection with blood samples obtained serially. Total growth hormone concentration is measured by radioimmunoassay; components of circulating growth hormone are measured by gel chromatography and radioimmunoassay.

Progress and Results: Four patients have been studied. Because of a re-assignment of research priorities, the chromatographic studies have not been performed thus far. In addition, TRH has not been available for further studies. Therefore, the data are incomplete and insufficient to draw any conclusions.

Conclusions: None

Funds Utilized FY-76: None

Funding Requested FY-77: None

Publications (FY-76): None

Type of Report: Interim

Work Unit No. 1337

Title of Project: Growth Hormone Responses to TRH in Acromegaly

Investigators:

Principal: LTC Richard C. Dimond, MC

Associates: LTC D. Corrigan, LTC L. Wartofsky, M. Schaaf, M.D.,  
and COL J.M. Earll

Objective : Assess the inhibitory effects of thyroid hormone, glucose  
and L-Dopa on the abnormal growth hormone response to  
TRH in acromegaly.

Technical Approach: Standard TRH stimulation tests after administration  
of thyroid hormone, during a constant infusion of  
glucose, and after the administration of L-dopa.

Progress and Results: Five patients have been studied, but complete  
data are available in only three patients. The  
preliminary data from these three patients suggest  
that thyroid hormone, glucose, and L-Dopa do  
not block the abnormal growth hormone response to  
TRH in acromegaly. Unavailability of TRH has  
precluded studying additional patients. The  
data are incomplete and insufficient to draw  
any conclusions.

Conclusions: None

Funds Utilized FY-76: None

Funding Requested FY-77: None

Publications (FY-76): None

Type of Report: Interim

Work Unit No.: 1338

Title of Project: Hormonal and Metabolic Changes in Hypertension

Investigators:

Principal: Jerry M. Earll, M.D.

Associate: Marcus Schaaf, M.D.

Objectives: Normal and low Renin group of hypertensive patients would be studied metabolically to determine if there were any alterations of body composition suggestive of "Unidentified" mineral corticoid substances.

Technical Approach: Hypertension patients would receive a standard workup with careful screening to categorize them as to whether they were normal or low renin groups. The low renin patients were to be matched carefully by age to a normal renin hypertension patient. Whole body composition with emphasis on potassium determinations in a whole body counter were to be made. Five hypertension patients have been studied. Difficulty has been encountered in obtaining the low renin hypertension group for study. Six patients had basal prolactin levels studied while on low salt and high salt diets and following diuretic stimulation. There has been no significant change in basal prolactin during these manipulation.

Conclusion: Inspite of some animal evidence to indicate that prolactin may be an important hormone in manipulating salt and water metabolism, major changes in sodium intake have failed to stimulate or suppress prolactin. In addition, therapy with a potent diuretic has failed to stimulate prolactin.

Funds Requested: These will be provided by the general funding of the metabolic unit.

Publications: None

Type of Report: Interim

Work Unit Number: 1339

Title: Effect of Lithium on Intrathyroidal Iodine Content

Investigators: Timothy M. Boehm, MAJ MC

Objective: To ascertain whether chronic lithium therapy in psychiatric patients alters intrathyroidal iodine.

Technical Approach: To utilize the fluorescent scanner to measure intrathyroidal iodine content in patients receiving lithium therapy.

Progress: Preliminary studies have been completed in 3 patients; apparently some patients receiving chronic lithium have elevated intrathyroidal iodine. The pace of the study has been slowed because of the geographic separation of Forest Glen from the Main Hospital and limited availability of psychiatric patients.

Conclusions: None

Funds Utilized FY-76: None

Funding Requested FY-77: None

Publications (FY-76): None

Type of Report: Interim

Work Unit No.: 1341

Title of Project: Prolactin Levels in Patients Receiving Medicine for Hypertension.

Investigators:

Principal: Jerry M. Earll, M.D.

Associates: Marcus Schaaf, M.D. and Gordon L. Noel, M.D.

Objectives: Since reserpine has been associated with a significant increase of risk of developing human breast cancer, prolactin measurements were to be obtained in patients taking rawolfia derivatives for hypertension.

Technical Approach: Patients receiving reserpine from the pharmacy would be given an information sheet which would offer them the opportunity to have a blood specimen drawn at the metabolic department if they wished. A second specimen would be drawn two hours following the patients usual dose of medication. It was felt that this would permit comparison of basal levels of prolactin and any changes in prolactin following the acute dosage of medication.

Progress and Results: Two hundred information sheets were dispensed from the pharmacy and only four patients responded for the study. There was no tendency for an acute increase of serum prolactin following the ingestion of the usual dose of reserpine, nor did the basal levels of prolactin in this small group seem to be abnormal.

Conclusions: If there are abnormalities of prolactin secretion in patients taking rawolfia drugs for hypertension, much more sophisticated studies with multiple sampling every hour throughout the day or even including sleep studies on an in-patient basis would be necessary to identify them.

Funds Utilized: None

Funding Requested FY 77: None

Publications: None

Type of Report: Termination. It is believed any changes in prolactin secretion during hypertension medication may be quite subtle. The out-patient clinic approach to this problem does not appear to be a productive one and

any studies of prolactin responses to medication will be conducted on an in-patient basis under study protocol work unit number 1338 "hormonal and metabolic changes in hypertension." This project should be terminated at this time.

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Work Unit Number 1342

Title of Project: Dietary Influence on Prolactin Secretion

Investigators:

Principal: Jerry M. Earll, COL, MC

Associates: Marcus Schaaf, M.D., Kenneth Burman, MAJ, MC

Objectives: To evaluate changes in hormone secretion during alterations of carbohydrate and fat in the diet. Emphasis is being placed upon prolactin, growth hormone and thyroid hormone changes.

Technical Approach: Obese patients will be admitted to the metabolic ward where baseline studies will be performed prior to a high fat and low carbohydrate diet. Following these dietary manipulations, some patients will go through fasting episodes.

Progress and Results: Considerable delay occurred in obtaining final approval of this protocol from Office of the Surgeon General. This was recently received and 2 patients have now entered the study.

Conclusions: No laboratory data has been completed yet.

Funding Requirements: None

Publications: None

Type of Report: Interim

Work Unit No.; 1345

Title of Project: Conversion of Testosterone to Estradiol

Investigators:

Principal: Kenneth D. Burman, M.D., MAJ, MC

Associate: J. MacIndoe, M.D., MAJ, D. Lynn Loriaux, M.D.,  
J.M. Earll, M.D., COL, L. Wartofsky, M.D., LTC

Objectives: To determine testosterone conversion.

Technical Approach: Administration of radioactive testosterone and  
estradiol.

Progress & Results: No patients studied as yet.

Conclusions: None

Funds Utilized, FY-76: None

Funding Requirements, FY-77: None

Publications: None

Type of Report: Interim (Annual).

Work Unit No.: 1346

Title of Project: Thyroid Function Tests in Cord Blood, Maternal Sera and Amniotic Fluid.

Investigators:

Principal: Kenneth D. Burman, M.D., MAJ, MC

Associate: J. Read, MAJ, W. Patow, COL, F.D. Wright,  
L. Wartofsky, LTC.

Objectives: To determine if thyroid disease can be diagnosed in utero

Technical Approach: Amniotic fluid measurements.

Progress & Results: Samples on 21 patients have been collected and are now being analyzed and the results interpreted.

Conclusions: None yet.

Funds Utilized, FY-76: None

Funding Requirements, FY-77: None

Publications: Paper presented at annual meeting of American College of Obstetrics and Gynecology, Dallas, Texas, May 1976.

Type of Report: Interim (Annual).

Work Unit No.: 1347

Title of Project: Investigations into the Physiology of L-Reverse T<sub>3</sub> (rT<sub>3</sub>) and L-3,3'-Diiodothyronine

Investigators:

Principal: Kenneth D. Burman, M.D., MAJ, MC

Associate: D. Strum, M.D., MAJ, F.D. Wright, Jerry M. Earll, M.D., COL, L. Wartofsky, M.D., LTC

Objectives: To investigate the normal physiology of reverse T<sub>3</sub> and T<sub>2</sub>, products of T<sub>4</sub> and T<sub>3</sub> metabolism.

Technical Approach: Administration of rT<sub>3</sub> and 3,3'<sup>I</sup>T<sub>2</sub>

Progress & Results: 20 subjects have been studied. This material appear to have very short half-lives and rapid metabolic clearance rates.

Conclusions: Pending analysis of data.

Funds Utilized, FY-76: None

Funding Requirements, FY-77: None

Publications: None

Type of Report: Interim (Annual)

Work Unit Number: 1348

Title of Project: Correlation of Dose and Duration of Exogenous Steroid Therapy with Recovery of Hypothalamic-Pituitary-Adrenal Function

Investigators:

Principal: Timothy M. Boehm, MAJ MC

Associate: Joseph Bruton, Ph.D.

Objectives: To investigate the effect of high dose steroid therapy of less than 1 month duration in inducing hypothalamic-pituitary suppression, as reflected by ACTH responsiveness.

Technical Approach: To use metyrapone and insulin tolerance testing, with measurement of plasma cortisol and ACTH, as a measure of hypothalamic-pituitary suppression.

Progress and Results: Preliminary studies have been completed in one patient, and laboratory verification of the ACTH assay is pending.

Conclusions: None, presently

Funds Utilized FY-76: None

Funding Requirements, FY-77: None

Publications: None

Type of Report: Interim

Work Unit No.: 1402

Title of Project: Comparison of Endoscopic and Radiologic Evaluations of the Upper Gastrointestinal Tract.

Investigators:

Principal: Richard C. Cammerer

Associate: H. Worth Boyce, Jr.

Objectives: To determine the true diagnostic accuracy of endoscopy compared to upper gastrointestinal radiography.

Technical Approach: Patients with symptoms referable to the upper gastrointestinal tract were included in our study. The diagnostic yields of the panendoscopic (esophagogastroduodenoscopy) examination and the upper gastrointestinal series were compared prospectively. The radiographic findings were withheld from the endoscopist and the endoscopic findings were withheld from the radiologist. The findings from each study were recorded, categorized, tabulated and compared on the basis of percentage of accurate diagnosis by both procedures and by each procedure independently.

Progress and Results: Two hundred patients have been included in the study and the data has been tabulated and compared.

Conclusion: This study has demonstrated that endoscopy is superior to X-Ray in defining most upper gastrointestinal lesions. Additionally, the efficacy of the endoscopic examination of the esophagus, stomach and duodenum is independent of the radiographic evaluations and should be used in the evaluation of symptomatic patients even when the X-Ray studies appear to be normal.

Funds Utilized: None.

Funding Requirements: None.

Publications: A manuscript to be submitted for publication is being prepared. The results from this study were presented at the William Beaumont Gastrointestinal Symposium, March 1976, by the principal investigator.

Type of Report: Completed.

Work Unit No.: 1404

Title: Cholestyramine in the Treatment of Peptic Disorders.

Principal Investigator: David Staples, M.D.,  
Department of Medicine  
Gastroenterology Service

Status: Several requests for the Annual Progress Report on this project have been ignored. The Clinical Investigation Committee in a meeting on 29 September 1976, terminated this study.

Work Unit Number: 1405

Title: Intraluminal Dynamics in Malabsorption

Investigators: H. Worth Boyce Jr., M.D., COL, MC

Objective: To evaluate intraluminal digestion in a group of patients with various types of malabsorption.

Technical Approach: A small intestinal tube is fluoroscopically passed to a point 20 cm beyond the ligament of Treitz. Each patient then drinks a 300 ml test meal consisting of 74 gms corn oil, 126 gms skimmed milk powder, 138 gms dextrose homogenized in water to a volume of 1000 ml. The intestinal contents are collected by gravity drainage in four 30-minute aliquots and immediately frozen in a dry-ice acetone bath and stored at -20° C until the time of analysis. One half of each aliquot is quickly thawed and then heated to 70° C for 10 minutes to inactivate pancreatic lipase before chemical analysis. The following chemical determinations are performed on each of the heated aliquots:

- a. Total lipids (gravimetric method of Hofmann).
- b. Total fatty acid (titration method of Dole).
- c. Micellar lipid (ultracentrifugation, and gravimetric method of Hofmann).
- d. Total bile salts (enzymatic dehydroxylation method of Admirand and Small).
- e. Micellar bile salts (ultracentrifugation and enzymatic dehydroxylation method of Admirand and Small).
- f. Qualitative differentiation of bile salts--conjugation vs. unconjugation (thin-layer chromatography utilizing the solvent system of Hofmann).
- g. pH (routine method).

Progress and Results: Each of the chemical terminations listed above has been set up and evaluated using standard solutions. Five normal patients have been studied, and all test results were comparable with those reported in the literature. Patients were assessed into the protocol, however, because of numbers involved, no significant results have occurred. This protocol, in all likelihood, will be continued by the principal investigator who left the service in July 75 and is currently located at USF Medical Center, Tampa, Fla.

Work Unit #1405 Con't

Conclusion: No definitive conclusions are yet available because of study population size.

Fund Utilized FY 76: None.

Funding Requested FY 77: None.

Publications: None to date.

Type of Report: Terminated as principal investigator has retired from the Army and is planning to undertake this at his new position.

Comment: The technician, equipment, and lab area have all been utilized to accomplish the following published reports:

1. "The Relationship of Bile in the Stomach to Gastritis"  
F. H. Goldner, H. W. Boyce, Gastrointestinal Endoscopy  
22:197-199, May 76.
2. "The Occurance of Bacteremia After Esophageal Dilation",  
D. R. Raines, W. C. Branche, D. L. Anderson, H. W. Boyce,  
Gastrointestinal Endoscopy 22:86-87, Nov 75.

The technician is currently funded in protocol #1408 as well as #1413.

PROTOCOL #1408

TITLE: Bile Salt Clearance in Chronic Active Hepatitis

Department of Gastroenterology, Division of Medicine

PRINCIPAL INVESTIGATORS:

Principal Investigators: Lawrence F. Johnson, M.D., LTC MC

Edgar C. Boedeker, M.D., MAJ, MC

Co-investigators: M. Dean Kinsey, M.D., MAJ, MC

Rowen K. Zetterman, M.D., MAJ, MC

MANAGEMENT DATA: Project 3A672760A822, In-House Independent Research

Task 00

Work Unit 150 Gastrointestinal diseases of military  
importance

OBJECTIVE: To ascertain if the clearance rate of intravenously administered cholyl glycine (a) can reliably determine those patients with chronic active hepatitis who require corticosteroid therapy; and (b) can determine the endpoint of such therapy in those patients who are treated.

Technical Approach.

(a) Development of radioimmunoassay (RIA) for conjugates of cholic acid.

- (1) Antibody to cholyl glycine will be developed in rabbits by linking cholyl glycine covalently bound to bovine serum albumin by the carbodiimide method (9). After separating the free from the bound bile acid by dialysis the albumin-cholyl glycine complex is emulsified with an equal volume of Freund's complete adjuvant and injected into rabbits at weekly intervals. Antibody titers will be assessed at two month or until an adequate titer is obtained for RIA.
- (2) Assay (10): The assay is performed on 0.1 ml aliquots of unextracted serum. The reaction mixture contains: 0.1 ml of human gamma globulin diluted (1:25 v/v dilution); 0.1 ml of  $^{3}\text{H}$ -cholyl glycine ( $10^5\text{cpm}$ ); 0.1 ml of unlabeled cholyl glycine (to prepare standard curves) or unknown serum for bile acid assay; 0.1 ml of antibody to cholyl glycine (1:60 titer); and 0.01M potassium phosphate buffer, pH 7.4 to a final volume of 1.0 ml. The mixture is incubated for 1 hour at  $37^{\circ}\text{C}$ , and then placed at  $4^{\circ}\text{C}$  for 10 minutes. The free antigen is separated from bound antigen by polyethylene glycol and 1 ml of the supernatant decanted into scintillation vials containing 10 ml of Hydromix for counting. Each human serum sample will be run in triplicate with simultaneous determination of a known standard curve.

(b) Patient selection:

- (1) Controls: A group of 30 healthy volunteers with no apparent liver disease will establish the range of normal cholyl glycine clearance. Informed consent will be obtained.
- (2) Patients: All patients in whom the diagnosis of chronic active hepatitis, minimal change type, is established by clinical and histologic parameters will be entered into the study. Informed consent will be obtained. Each patient will have an evaluation of cholyl glycine clearance before initiation of therapy which will be repeated at 6 month intervals, in conjunction with routine clinical and histologic follow-up, until histologic remission occurs.

(c) Cholyl glycine clearance: Following an overnight fast and a baseline 2 cc blood sample for cholic acid determination, cholyl glycine (5 M/kg) will be administered intravenously. From another vein, 2 cc blood samples will be drawn at 1 minute intervals for 10 minutes and then at 5 minute intervals for an additional 20 minutes (total blood sample - 30 cc's). A disappearance curve will then be constructed and the half-life of intravenously administered cholyl glycine determined.

The clearance rate of cholyl glycine will be related to the clinical and histologic findings at each evaluation. The relationship of cholyl glycine half-life to both response to corticosteroid treatment and to histologic status will be determined. It is anticipated that this liver function test will be capable of determining those patients who will require corticosteroid therapy. Furthermore, in group so treated, it should be capable of determining the endpoint of such therapy. This may obviate the need for repetitive liver biopsy and thus reduce hospitalization time and expense.

Progress and Results.

The work to date has consisted primarily of development of the radioimmunoassay and preliminary toxicity and safety testing of the cholyl glycine to be used intravenously.

Antibody to cholyl glycine was developed in rabbits injected with cholyl glycine - BSA conjugate. One rabbit (#802) developed an antibody of adequate titer (1:60) to develop and validate the radioimmunoassay. This rabbit, however, died after 2 booster injections and 2 bleeds. Adequate amounts of antibody were stored at -20 C and the assay was developed. Standard curves and fasting levels in normal volunteers were determined. Concurrent with development of a cholyl glycine antibody an additional set of rabbits were injected with glycochenodeoxycholic acid - BSA conjugate. One rabbit (#837) developed an antibody to glycochenodeoxycholic acid of adequate titer (1:250) to develop an assay.

After preliminary development, normal values for conjugates of cholic and chenodeoxycholic acid were determined in a group of normal volunteers. At this point, a series of mechanical and power failures destroyed the antibody to conjugates of cholic acid. Additional rabbits were injected in an effort to regain an adequate antibody. Work continued on the development of this antibody to conjugate of chenodeoxycholic acid and serum sample from a group of patients with hyperthyroidism were assayed. These patients were being studies under a separate protocol (#1411) to determine if alterations in bile acid metabolism could explain the pruritus observed in a significant number of hyperthyroid patients.

Concurrently with the studies on development of the assay, preliminary studies were conducted to obtain a safe intravenous preparation of cholyl glycine for human use. Rabbits were injected intravenously with 10 cc of a cholyl glycine solution containing 50 mol/cc. This is the approximate dose for a 100 kg human. In a rabbit, this represents about 35-50 times the dose in humans. At this dose, approximately 20% of the rabbits died. Further studies are planned using a dose closer to the rabbit equivalent.

#### Conclusions.

Although the primary goal of development of a radioimmunoassay achieved only limited success, a good assay was developed for conjugates of chenodeoxycholic acid. With this antibody, we were able to define abnormalities of bile acid metabolism in hyperthyroidism. Developmental work on the antibody for conjugates of cholic acid continues and appears promising.

#### Publication.

- (1) Kinsey, M.D., Donowitz, M., Boehm, T., et al: The correlation between pruritus and elevated serum chenodeoxycholic (CDC) acid levels in hyperthyroidism. Gastroenterology 70: A44, 1976.

#### Type of Report - Interim.

## FUNDING IMPLICATIONS:

YEARLY TOTAL

(a) Personnel:	
Chemist - Corinne Maydonovitch	\$15,482.00
(b) Equipment:	
No additional equipment needed	
(c) Consumable Supplies:	
(1) Rabbits #20 @ \$20.00	400.00
(2) $^3\text{H}$ -cholyl glycine (New England Nuclear) 1.5 mC at \$104.00 per 250 uC	624.00
(3) Scintillation vials #9 cases case of 500 @ \$45.00	405.00
(4) Hydromix #12 gallon gallon @ \$36.00	432.00
(5) Glycocholic acid 50 grams 25 grams @ \$160.00	320.00
(6) Eppendorf Pipette Tips #5000 1000 @ \$48.00	240.00
(7) Animal Maintenance \$.33/day Average 6 rabbits maintained/year	712.80
(d) Travel to present paper (TDY):	550.00
(e) Consultation Fees:	1,000.00
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	\$18,165.80

Work Unit No.: 1410

Title: Percutaneous (blind) vs. laparoscopic (direct vision) liver biopsy in assessing chronic active hepatitis.

Investigators: Rowen K. Zetterman, Maj., M.C.  
Richard C. Cammerer, Maj., M.C.  
Lawrence F. Johnson, LTC, M.C.

Objective: To ascertain if the diagnosis and management of chronic active hepatitis can be adequately monitored by percutaneous (blind) liver biopsy or whether laparoscopic (direct vision) liver biopsy should be utilized.

Technical Approach: Patients with chronic active hepatitis are currently evaluated by percutaneous liver biopsy. This means of biopsy often fails to establish the presence of cirrhosis. Under the guidelines of this study, patients with chronic active hepatitis who have equivocal percutaneous biopsies will be further evaluated by laparoscopy with biopsy under direct vision, and the combined diagnostic accuracy of observation and directvision biopsy will be compared to that of percutaneous biopsy alone. This will establish the best method of diagnosis and management for our patients with chronic active hepatitis.

Progress: To date, three patients have been accessioned to the study. In two patients, percutaneous biopsy was as accurate as laparoscopy in defining the presence of extensive fibrosis without cirrhosis. However, one patient had cirrhosis established only by laparoscopy with direct vision biopsy.

Conclusion: We simply need to continue to evaluate all appropriate patients until an adequate number for statistical significance have been accessioned.

Funds Utilized: None.

Funds Requested FY-77:	Travel	\$400.00
	Publication	200.00

Publications (FY-76): None.

Type of Report: Interim.

Addendum: Please note that for FY-77, the principal investigators should be changed to:

Howard Heit, Maj., M.C.  
Lawrence F. Johnson, LTC, M.C.

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Work Unit No.: 1501

Title of Project: ALGB Protocol #7111 - Add. #7: Vincristine - prednisone - Dexamethasone (induction) and 6-mercaptopurine - BCNU (intensification) with CNS treatment in untreated acute lymphocytic leukemia in patients under 20 years.

Investigators:

Principal: Johannes Blom, M.D.

Associate: Frederick B. Ruymann, M.D., LTC, MC

Objectives: 1. To determine whether dexamethasone is at least equal to prednisone in its antileukemic activity.

2. To determine whether dexamethasone decreases the recurrence of infection and particularly fatal sepsis during the induction phase.

3. To determine in untreated children if the frequency of remissions obtained with vincristine and corticosteroids can be increased by the addition of L-asparaginase and whether the position of L-asparaginase in the treatment schedule (before, during, or after the vincristine and steroids) influences the outcome.

4. To determine in untreated children if the duration of remission is influenced by the use of L-asparaginase in induction and whether the position of L-asparaginase in the treatment schedule (before, during, or after the vincristine and steroids) influences the remission duration.

5. To determine the remission duration achieved with prolonged methotrexate and 6-MP and BCNU in repeated intensive courses interspersed with periodic inducer doses of vincristine and steroids.

6. To determine whether the occurrence of CNS leukemia can best be forestalled by use of intensive intrathecal methotrexate or the combination of radiotherapy to the cranium and intrathecal methotrexate.

7. To evaluate through direct and historical comparison of the best prior studies of ALGB the effect of intensive prophylactic CNS therapy upon the overall remission duration.

8. To prepare patients with intensive systemic and CNS therapy for further therapy of chemotherapeutic or immunologic nature to be specified, which will commence after one year of maintenance treatment.

Technical Approach: Induction - Regimen 1: vincristine 2 mg/M<sup>2</sup> IV weekly plus prednisone 40 mg/M<sup>2</sup>/day or dexamethasone 6 mg/M<sup>2</sup>/day

Regimen 2: with prednisone or dexamethasone, L-asparaginase 1000 IU/kg/day IV daily for ten days either before, during, or after the vincristine - prednisone or dexamethasone.

Upon reaching complete remission, the patients are randomized between two intensification regimens, during this phase the patients randomly receive radiation therapy 2400 rads to the brain plus intrathecal methotrexate 12 mg/M<sup>2</sup> or methotrexate intrathecally only without radiation to the brain.

Progress & Results: WRAMC entered 17 patients, 12 of these obtained a complete remission, one patient had to be removed from protocol while in complete remission, eight patients have relapsed, three are still in remission, varying from 1154 to 1834 days. One patient had a partial remission and relapsed on day 372, two patients did not obtain remission and two patients died during the induction phase on day 2 and 3.

The Acute Leukemia Group B entered 644 patients. The most recent analysis was in March 1976. Eighty-five percent of 639 evaluable patients achieved complete remission, with no significant effect upon that outcome attributable to the steroid used or the addition of ten days of L-asparaginase before, during, or after therapy with vincristine and steroid.

The study was closed to entry on 18 April 1974. The three patients who are still on study at WRAMC continue to be followed and reported to the Acute Leukemia Group B according to standard Group practice. This constitutes the final report.

- Conclusions:
- 1) L-asparaginase given before, during or after treatment with vincristine and steroids during the remission induction did not contribute to the response. However, remissions are significantly prolonged when L-asparaginase is given after the induction with vincristine and prednisone.
  - 2) There is no difference between prednisone or dexamethasone induction as far as induction rate or sepsis is concerned.
  - 3) There seems to be a lesser incidence of CNS involvement in patients who receive dexamethasone than in those who receive prednisone.

This was presented at the 66th Annual Meeting of the American Association of Cancer Research.

Funding Requirements: N/A

Publications: American Association of Cancer Research Proceedings, 66th Annual Meeting, May 7-11, 1975, Abstract 730, page 183.

Work Unit No.: 1502

Title of Project: ALGB Protocol #7481 - Add. #0: Effect of long term surgical adjuvant systemic chemotherapy in mammary carcinoma; a comparative study of cyclophosphamide (NSC 2627), vincristine (NSC 67574), methotrexate (NSC 740), 5-fluorouracil (NSC 19893), prednisone (NSC 10023) vs. observation.

This protocol was discontinued on 15 May 1975 and replaced by Protocol #7581. No patients were entered into this study at WRAMC. This, therefore, constitutes the final report.

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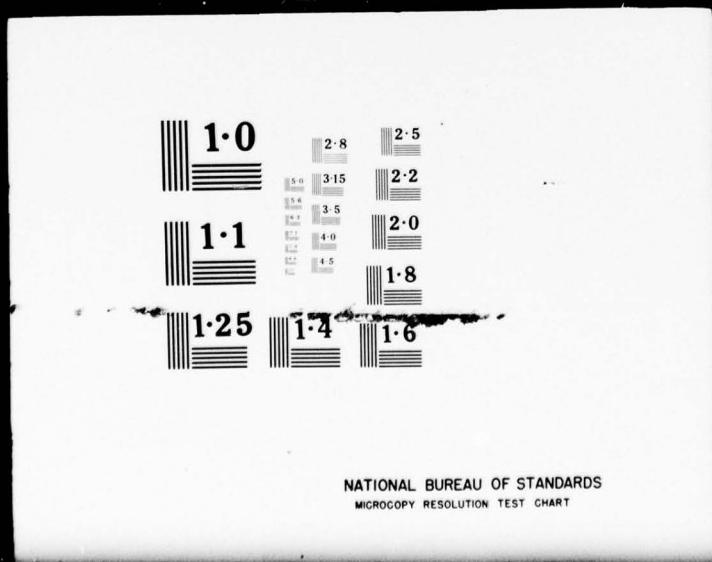
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Work Unit No.: 1506

Title of Project: ALGB Protocol #6801-S - Add. #0: Combination of L-asparaginase, prednisone, vincristine and daunorubicin in the treatment of acute, lymphoblastic leukemia - reinduction regimen.

Investigator:

Principal: Johannes Blom, M.D.

Objective: The objective of the L-asparaginase supplement is to study the use of L-asparaginase in combination with vincristine and prednisone with or without daunorubicin. To determine whether it may lead to greater remission duration and survival time than in the instance where the standard induction agents are utilized alone.

Technical Approach: The patients are randomized between induction with L-asparaginase or no L-asparaginase. Patients who receive no L-asparaginase are then randomized between vincristine-prednisone and vincristine-prednisone-daunorubicin induction. Patients who receive L-asparaginase first are subsequently randomized between the two induction regimens. Maintenance and intrathecal methotrexate are as in protocol #6801.

Progress & Results: WRAMC entered 6 patients; four obtained a complete remission, one is still in remission on day 2307, one relapsed on day 1150, one on day 449 and one on day 207. One patient had a partial remission, and one died on day 4 during the induction from hemorrhage of esophageal ulcers.

The Group entered 294 evaluable patients; 89% achieved a bone marrow remission, 8% died during induction. There were no significant differences between the induction regimens. As in protocol #6801, 6-MP plus methotrexate was better than methotrexate alone, and reinforcement with monthly vincristine-prednisone is not significantly different from vincristine-prednisone-daunorubicin. Prophylactic intrathecal methotrexate significantly reduces the rate of development of central nervous system leukemia.

This study was closed to entry of new patients on 5 February 1971. This constitutes the final report. The one patient who remains on study will

continue to be followed and reported to the ALGB according to standard group practices.

- Conclusions:
1. Combination 6-MP plus weekly methotrexate is more effective for maintenance than twice weekly methotrexate.
  2. Intrathecal injections of methotrexate are of major benefit in prolonging remissions when given before the appearance of clinical CNS leukemia.
  3. Beneficial effects of reinforcement treatments with vincristine-prednisone are diminished if daunorubicin is added to the reinforcement treatment.
  4. L-asparaginase administered for five days prior to vincristine and prednisone induction treatment demonstrated no beneficial effects upon either the frequency or the duration of complete remission.
  5. Approximately 25% of patients continue to be in complete remission 6-1/2 years from onset.

Funding Requirements: N/A

Publications: None.

Work Unit No.: 1507

Title of Project: ALGB Protocol #7021 - Add. #1: Combination chemotherapy of acute myelocytic leukemia (all ages) with two dose levels of arabinosyl cytosine (Ara-C) + thioguanine (TG).

Investigators:

Principal: Johannes Blom, M.D.

Objectives: 1. To reinvestigate the remission induction effects of Ara-C + TG when the former is given as a one hour infusion at the dose previously used in protocol #6822.

2. To compare the above with the effects on remission and survival of 50% of the initial dosage of Ara-C + TG.

Technical Approach: Regimen 1: Ara-C 100 mg/M<sup>2</sup> IV daily in a one hour drip plus TG 2.5 mg/kg/day divided into two oral doses.

Regimen 2: Ara-C 50 mg/M<sup>2</sup> IV daily in a one hour drip plus TG 1.25 mg/kg/day divided into two oral doses.

Maintenance Regimen: TG 2.5 mg/kg/day by mouth plus Ara-C 30 mg/M<sup>2</sup> subcutaneously once weekly.

Progress & Results: WRAMC entered nine patients, two obtained a complete remission, one patient is still in remission on day 1893, one relapsed on day 512, two had no response, one died from sepsis and hemorrhage on day 16, one died on day 34 from respiratory arrest, one died on day 4 from sepsis, bilateral pneumonia, and central nervous system leukemia, one died on day 39 from sepsis, and one died with a WBC of 209,000 from a cerebral hemorrhage.

The ALGB entered 273 patients, 230 patients were evaluable. Complete and partial remission for those who received full dose induction was 35%, for those who received half dose induction 23%. The median survival of patients who obtained a complete remission is approximately 14 months, while the median survival of patients who obtained a partial remission is approximately eight months. The median survival in non-responders is approximately one month. This study

was closed to entry of new patients on 21 June 1971. The one patient who was still on study at WRAMC will continue to be followed and reported to ALGB according to standard Group practice. This constitutes the final report.

**Conclusions:** The frequency of complete remissions and of complete plus partial remission is significantly greater for the high dose, although the duration of remission is of equal length whether obtained with full dose or half dose.

**Funding Requirements:** N/A

**Publications:** None.

Work Unit No.: 1508

Title of Project: ALGB Protocol #7121 - Add. #0: Comparison of intermittent cytosine arabinoside plus thioguanine with cytosine arabinoside plus CCNU (1,)2-chloroethyl)3-cyclohexyl-1-nitrosourea in induction and maintenance of untreated acute myelocytic leukemia.

Investigators:

Principal: Johannes Blom, M.D.

- Objectives:
1. To compare the remission induction effectiveness of an intermittent dose schedule Ara-C plus TG with Ara-C plus CCNU.
  2. To compare remission maintenance by continuation of the induction treatment on a modified schedule.
  3. To compare survival of patients treated by either of the two experimental regimens.
  4. To compare the data achieved in this study with that in the next study, 7122.

Technical Approach: Induction

Regimen 1: Ara-C 100 mg/M<sup>2</sup> IV daily in a 24-hour continuous infusion for five days plus thioguanine 100 mg/M<sup>2</sup> twice daily p.o. for five days.

Regimen 2: Ara-C 100 mg/M<sup>2</sup> IV daily in a 24-hour continuous infusion for five days plus CCNU 100 mg/M<sup>2</sup> p.o. on day 1.

Each five day course is repeated after a nine day rest period.

Maintenance

Regimen 1: Ara-C 100 mg/M<sup>2</sup> IV q 12 hours x 10 for five days plus thioguanine 100 mg/M<sup>2</sup> q 12 hours x 10 p.o. five days.

Regimen 2: Ara-C 100 mg/M<sup>2</sup> IV q 12 hours x 10 for 5 days plus CCNU 100 mg/M<sup>2</sup> p.o. on the first day of every other course.

These maintenance courses are repeated every 28 days until relapse.

**Progress & Results:** WRAMC entered seven patients, four obtained a complete remission, all of whom have now relapsed.

The Group entered 145 patients, 130 of whom were evaluable. Complete remission obtained with Ara-C and 6-TG was 26% and complete plus partial remission 45%. Complete remission obtained with Ara-C plus CCNU was 31% and complete plus partial remission was 38%.

Contrary to other studies, the best response was in the 40 to 59-year-old group. Percent of patients surviving and percent of patients remaining in remission is statistically not significantly different at the present time. The study was closed to entry of new patients on 31 December 1971. Since all patients entered on study at WRAMC have now relapsed, this constitutes the final report.

**Conclusions:** There is no statistical difference between the two induction regimens.

**Funding Requirements:** N/A

**Publications:** None.

Work Unit No.: 1510

Title of Project: ALGB Protocol #7501 - Add. #1: The treatment of lymphosarcoma and reticulum cell sarcoma.

Investigators:

Principal: Johannes Blom, M.D.

- Objectives:
1. To determine whether the dose of vincristine in induction is related to the frequency of complete remission and/or the length of remission time.
  2. To determine the remission duration of infrequent high doses of cyclophosphamide compared to daily cyclophosphamide.
  3. To determine whether reinduction with vincristine and prednisone prolongs the remission time in patients who are in continuous remission.

Technical Approach: Induction

Regimen 1: vincristine 20 mcg/kg IV q week plus prednisone 1 mg/kg/day p.o.

Regimen 2: vincristine 40 mcg/kg/week IV plus prednisone 1 mg/kg/day p.o.

Maintenance

Regimen A: cyclophosphamide 2 mg/kg/day p.o.

Regimen B: cyclophosphamide 2 mg/kg/day p.o. plus vincristine 20 or 40 mcg/kg IV q four weeks plus prednisone 1 mg/kg/day seven days q four weeks.

Regimen C: cyclophosphamide 30 mg/kg IV q four weeks.

Regimen D: cyclophosphamide 30 mg/kg q four weeks plus vincristine 20 or 40 mcg/kg q four weeks plus prednisone 1 mg/kg/day for seven days q four weeks.

**Progress & Results:** WRAMC entered 11 patients, six had a complete remission. Although one patient is still in complete remission at almost six years, this patient's treatment has deviated from the protocol, and he was, therefore, removed from study. One patient, who had a complete remission, is lost to followup, and one patient, who had a complete remission, had a myocardial infarction and died on day 505. One patient with a complete remission had progressive disease on day 1478, one on day 666, and one on day 95. Three patients had a partial remission and subsequently progressed, one patient had an improvement and subsequently had progressive disease on day 302, one had progressive disease on day 11.

The Group entered 460 patients, 358 of whom were evaluable. There does not seem to be a significant difference between high and low dose of vincristine induction regimens. The study was closed to entry of new patients in May 1972. Since there are no more patients on study at WRAMC, this constitutes the final report.

**Conclusions:** Induction responses are similar for 20 and 40 mcg/kg of vincristine per week plus prednisone daily. Vincristine-prednisone reinforcement is of benefit in lymphosarcoma but does not seem to be of benefit in reticulum cell sarcoma.

**Funding Requirements:** N/A

**Publications:** None.

Work Unit No.: 1511

Title of Project: ALGB Protocol #6604 - Add. #5: Intensive radiotherapy - chemotherapy study of generalized Hodgkin's disease.

Investigators:

Principal: Johannes Blom, M.D.

Objectives: To determine the effect of radiation therapy or chemotherapy alone, chemotherapy followed by radiation therapy, or radiation therapy followed by chemotherapy in patients with Stage III and selected cases with Stage IV Hodgkin's disease.

Technical Approach: Velban 0.1 mg/kg/week for four weeks followed by one dose nitrogen mustard 0.4 mg/kg, radiation therapy to all the lymph node bearing areas or to lymph node areas involved with Hodgkin's disease only.

Progress & Results: WRAMC entered 43 patients, two were Stage IV, seven patients received chemotherapy only, and all relapsed. Thirty-four patients received radiation therapy or a combination of radiation therapy and chemotherapy. Of these 34 patients, three were disqualified, two were lost to follow-up, and three died while on study. Of these three patients, two died from complications of the radiation therapy without any evidence of disease at autopsy, one at day 974 and the other at day 633. The third one died of progressive disease while receiving the radiation therapy, one had no response. Of the 25 patients who responded, thirteen have relapsed from 154 to 2359 days. Twelve patients are still in complete remission from 1676 to 2927 days.

The Group entered 148 patients, 101 were evaluable. Twenty-two patients were treated with chemotherapy alone, 19 responded and 18 relapsed; however, their survival thus far is indistinguishable from the other treatment regimens, presumably because they received radiation therapy after exit from protocol.

The most recently presented data were at the Annual Meeting of the American Society of Clinical Oncology in April 1974. The group that received

the chemotherapy followed by radiation therapy has the longest duration of remission, the median being 57 months. Median survival of all patients has now been reached at 54 months. In the adequately treated patients, none of the treatment groups have reached a median survival.

The study has been closed to entry of new patients. This constitutes, therefore, the final report. The patients who are still on study will continue to be followed and reported to the Acute Leukemia Group B according to standard Group practice.

**Conclusions:** It is important that in symptomatic patients chemotherapy before radiation therapy is given because it is significantly better.

**Funding Requirements:** N/A

**Publications:** Presented at a symposium on Hodgkin's Disease in St. Louis on 8 October 1971, published in the Archives of Internal Medicine, March 1973, pages 424-428. Presented at the 10th Annual Meeting of the American Society of Clinical Oncology, March 27-30, 1974, Abstract 706.

Work Unit No.: 1512

Title of Project: ALGB Protocol #6951 - Add. #3: Combination chemotherapy of generalized Hodgkin's disease.

Investigators:

Principal: Johannes Blom, M.D.

Objectives: 1. To demonstrate whether combination chemotherapy can be curative in Hodgkin's disease stages IIIB and IV.

2. To induce complete or partial remission in as high a percentage as possible.

3. To examine the relative value of four different combinations, among which are two 4-drug and two 3-drug programs, in remission induction.

4. To compare the relative value of three types of maintenance treatment.

5. To increase the survival time of patients with stage IIIB and IV Hodgkin's disease.

Technical Approach: Induction

Regimen I: vincristine 1.4 mg/M<sup>2</sup>/week x 2 IV  
plus  
prednisone 40 mg/M<sup>2</sup>/day x 14 p.o.  
(1st and 4th courses only)  
plus  
BCNU 80 mg/M<sup>2</sup>/week x 1 IV

Each course to be repeated every 28 days for a total of six courses.

Regimen II: vincristine 1.4 mg/M<sup>2</sup>/week x 2 IV  
plus  
prednisone 40 mg/M<sup>2</sup>/day x 14 p.o.  
(1st and 4th courses only)  
plus  
procarbazine 50 mg on day 1 p.o.  
100 mg on day 2 p.o.  
100 mg/M<sup>2</sup>/day on days 3-14 p.o.

Regimen III: vincristine 1.4 mg/M<sup>2</sup>/week x 2 IV  
plus  
prednisone 40 mg/M<sup>2</sup>/day x 14 p.o.  
(1st and 4th courses only)

plus  
procarbazine 50 mg on day 1 p.o.  
100 mg on day 2 p.o.  
100 mg/M<sup>2</sup>/day on days 3-14  
p.o.

plus  
BCNU 80 mg/M<sup>2</sup> IV on day 1

Regimen IV: vincristine 1.4 mg/M<sup>2</sup>/week x 2 IV  
plus  
prednisone 40 mg/M<sup>2</sup>/day x 14 p.o.  
(1st and 4th courses only)  
plus  
procarbazine 50 mg on day 1 p.o.  
100 mg on day 2 p.o.  
100 mg/M<sup>2</sup>/day on days 3-14  
p.o.  
plus  
nitrogen mustard 6 mg/M<sup>2</sup>/week x 2 IV

Maintenance

Regimen I: vinblastine 6 mg/M<sup>2</sup>/week IV for two months, after this every other week, until relapse.

Regimen II: chlorambucil 6 mg/M<sup>2</sup>/day p.o. until relapse.

Regimen III: chlorambucil 6 mg/M<sup>2</sup>/day p.o.  
plus  
vincristine 1.4 mg/M<sup>2</sup> x 1 IV and  
prednisone 40 mg/M<sup>2</sup>/day x 7 after this every other month until relapse.

Progress & Results: WRAMC entered 41 patients, 39 were evaluable, 21 had a complete remission, six patients are still in complete remission from 1696 to 2394 days after initiation of therapy. Three patients expired while still in remission from complications of treatment. Eleven patients had a complete response and relapsed from day 190 to day 1778. Five of these eleven patients have expired. Twelve had partial remission, all of whom have now relapsed. Five of these twelve have expired, seven relapsed from 175 to 2109 days. Two patients had progressive disease, three had an improvement and expired subsequently. One patient had no change and two were disqualified.

The Group entered 581 patients, 515 of whom were evaluable. Induction regimens 3 and 4 continued to be more effective. The complete remission rates in these four induction regimens are 40%, 47%, 63% and 59%, and the complete plus partial remission rates are 76%, 74%, 92% and 88%. The duration of response by induction regimen and maintenance regimen shows that the MOPP therapy and BOPP therapy are equal and compare favorably with the MOPP regimen in protocol #6712. It seems that the maintenance with chlorambucil and vincristine and prednisone reinduction is somewhat better than the other two treatment regimens. The last analysis was at the August 1973 meeting. At the December meeting, it was decided to discontinue the maintenance therapy after the treatment time of 3-1/2 years.

The study was discontinued on 10 March 1972. This constitutes the final report. Patients who are still on study will continue to be followed and will be reported to the ALGB according to standard Group practices.

**Conclusions:** Treatment with the 4-drug combinations is better than with the 3-drug combinations and the maintenance with chlorambucil and vincristine plus prednisone reinductions is better than chlorambucil alone or vinblastine.

**Funding Requirements:** N/A

**Publications:** None.

Work Unit No.: 1514

Title of Project: ALGB Protocol #7181 - Add. #1: Adriamycin (NSC 123127) and amputation in primary osteogenic sarcoma.

Investigators:

Principal: Johannes Blom, M.D.

Objectives: To determine the effectiveness of one year of repeated treatment with adriamycin in conjunction with radical amputation of the localized osteosarcoma in an attempt to decrease the recurrence rate and enhance the survival of patients with osteogenic sarcoma.

Technical Approach: Adriamycin 30 mg/M<sup>2</sup>/day IV for three consecutive days. The first four courses are given with six weeks interval and the following eight courses are given with eight weeks interval.

Progress & Results: WRAMC entered seven patients. One patient went off study on day 31 because he refused further therapy, one patient relapsed on day 548, and one patient relapsed on day 217. No recent information is available on one patient, three patients are still in complete remission from day 160 to day 1112.

ALGB entered 98 patients, 84 of whom were evaluable at the meeting in March 1976. The disease free period for patients who have followed protocol exactly is considerably longer than for historical controls, and also for patients who have had a protocol violation, although their disease-free period is also still longer than that of the historical controls.

The protocol was closed to entry of new patients on 24 February 1976. This constitutes the final report.

Conclusion: Adriamycin seems to be a valuable agent in preventing metastasis in osteogenic sarcoma after amputation of the primary lesion.

Funding Requirements: See introductory remarks to Annual Research Report.

Publications: Proceedings of the 10th Annual Meeting of the American Society of Clinical Oncology, March 27-30, 1974,  
Abstract 745.

New England Journal of Medicine, Vol. 291, 7 November  
1974, pages 998-1000.

Work Unit No.: 1516

Title of Project: ALGB Protocol #7291 - Add. #2: Intergroup rhabdomyosarcoma study; role of postoperative radiotherapy and combinations of dactinomycin, vincristine, cyclophosphamide and adriamycin in childhood rhabdomyosarcoma by Acute Leukemia Group B, Southwestern Cancer Chemotherapy Study Group and Childrens Cancer Study Group A.

Investigators:

Principal: Johannes Blom, M.D.

Associate: Frederick B. Ruymann, M.D., LTC, MC

- Objectives:
1. To determine whether postoperative radiotherapy prevents local recurrence and improves the survival rate after what appears to be complete surgical removal of the localized tumor.
  2. To compare duration of remission, recurrence and survival of patients treated with vincristine and dactinomycin with those treated with vincristine, dactinomycin and daily oral cyclophosphamide.
  3. To compare in terms of response to treatment, length of remission, percentage exhibiting recurrence and survival of the effectiveness of vincristine, dactinomycin and high pulse doses of cyclophosphamide to the same drug combination plus adriamycin for the treatment of patients with gross residual disease at the time of diagnosis.

Technical Approach: Patients are divided into four groups:

Group 1 - localized disease completely removed

Group 2 - grossly removed tumor with microscopic residual disease

Group 3 - incomplete removal of tumor or biopsy with gross residual disease

Group 4 - distant spread of disease present at onset

Patients are randomized according to their disease group and treatment started within 72 hours of surgery.

The patients in group 1 will be randomized between regimen A and B, patients in group 2 will be randomized between regimen C and D (regimen D is the same as regimen B), and patients in group 3 and 4 will be randomized between regimen E and F.

Regimen A: vincristine, 2 mg/M<sup>2</sup> (maximum dose 2.0 mg) IV weekly for 12 doses plus dactinomycin 0.015 mg/kg/day (max. 0.5 mg) IV for 5 days to be repeated 12, 24, 36 and 48 weeks plus cytoxan 2.5 mg/kg/day orally starting on day 42 and continuing it up through 24 months.

Regimen B: radiotherapy to the tumor bed after surgery plus chemotherapy as outlined in regimen A

Regimen C: radiotherapy to the tumor bed after surgery plus dactinomycin 0.015 mg/kg/day (max. 0.5 mg) IV for 5 days to be repeated at 9, 18, 27, 36 and 45 weeks plus vincristine 2 mg/M<sup>2</sup> (max. 2 mg) IV weekly for six doses

Regimen E: vincristine 2 mg/M<sup>2</sup> (max. 2 mg) IV weekly for 12 doses plus dactinomycin 0.015 mg/kg/day (max. 0.5 mg) IV for 5 days to be repeated 18, 30, 42 and 54 weeks plus cytoxan 10 mg/kg/day IV for 7 days, a second seven day course to be given by mouth at 13 weeks- cytoxan 2.5 mg/kg/day p.o. from 21st week through the 24th month of therapy plus radiotherapy to the tumor bed as well as to the areas of spread to be started at six weeks.

Regimen F: vincristine 2 mg/ $M^2$  (max. 2.0 mg)  
IV weekly for 12 doses plus  
dactinomycin 0.015 mg/kg/day (max.  
0.5 mg) in the vein for 5 doses  
to be repeated at 21, 33, 45 and  
57 weeks plus  
cytoxan 10 mg/kg/day IV for 7 days.  
A second 7 day course by mouth to  
be started at 13 weeks.  
cytoxan 2.5 mg/kg/day by mouth from  
the 24th week to the 24th month  
of therapy plus  
adriamycin 50 mg/ $M^2$  IV at 5, 18,  
27, 39 and 51 weeks. This will  
be reduced to 30 mg/ $M^2$  if a large  
bone marrow volume is to be  
irradiated (maximum total dose  
600 mg/ $M^2$ ) plus  
radiotherapy to the tumor bed as well  
as to the areas of spread to be  
started in six weeks.

Progress & Results: WRAMC entered one patient, who was followed for  
127 days and then lost to followup.

The Group entered 371 patients. The most recent  
report is of March 1976. The study continues.

Funding Requirements: See introductory remarks to Annual Research  
Report.

Publications: None.

Work Unit No.: 1517

Title of Project: ALGB Protocol #7331 - Add. #0: Hydroxyurea (NSC 32065), 6-mercaptopurine (NSC 755), and prednisone (NSC 10023) with or without vincristine (NSC 67574) and daunorubicin (NSC 84151) in the treatment of the resistant phase of chronic granulocytic leukemia. A phase III study.

Investigators:

Principal: Johannes Blom, M.D.

Objectives: 1. To assess the effectiveness of the combination of hydroxyurea, 6-MP and prednisone with or without vincristine in the resistant phase of chronic granulocytic leukemia for remission induction and maintenance.

2. To assess the effectiveness of daunorubicin as a consolidation agent.

Technical Approach:

Induction:

Regimen I - hydroxyurea 30 mg/kg/day p.o. in one dose  
plus  
6-MP 3 mg/kg/day p.o. in two divided doses  
plus  
prednisone 0.75 mg/kg/day p.o. in two divided doses.

Regimen II - hydroxyurea 30 mg/kg/day p.o. in one dose  
plus  
6-MP 3 mg/kg/day p.o. in two divided doses  
plus  
prednisone 0.75 mg/kg/day p.o. in two divided doses  
plus  
vincristine 1.5 mg/M<sup>2</sup> IV every week for four doses.

Consolidation:

Regimen A - daunorubicin 60 mg/M<sup>2</sup> daily for two days  
plus  
prednisone 0.25 mg/kg/day p.o. in two divided doses.

Regimen B - no consolidation.

Maintenance:

Regimen I - hydroxyurea 7 mg/kg/day p.o. one daily dose  
in a.m.  
plus  
6-MP 0.7 mg/kg/day p.o. one daily dose in a.m.  
plus  
prednisone 0.25 mg/kg/day p.o. in two divided  
doses

Regimen II - hydroxyurea 7 mg/kg/day p.o. one daily in a.m.  
plus  
6-MP 0.7 mg/kg/day p.o. one daily in a.m.  
plus  
prednisone 0.25 mg/kg/day p.o. in two divided  
doses  
plus  
vincristine 1.5 mg/M<sup>2</sup> in the vein once a  
month.

Maintenance phase will be continued until  
there is recurrence of disease.

**Progress & Results:** WRAMC has entered four patients. One patient was disqualified, one expired on day 39, and one expired on day 65. One patient went into complete remission, but had prolonged hypocellularity of the bone marrow. After recovery, she was placed on non-random maintenance regimen.

ALGB has entered 109 patients, 90 of whom are presently evaluable. At the most recent analysis in March 1976, complete and partial bone marrow remissions are 26% and 32% for the two regimens, and an overall response, which includes liver, spleen and lymph nodes, of 26% and 34%.

**Conclusion:** There is no difference between the two induction regimens and responses are unsatisfactory and rather short. Therefore, this protocol will be replaced by a new one as soon as approved.

**Funding Requirements:** See introductory remarks to the Annual Research Report.

**Publications:** None.

Work Unit No.: 1518

Title of Project: ALGB Protocol #7383 - Add. #0: Clinical trial of VP-16-213 (NSC 141540) (4'-dimethyl-epipodophyllotoxin-B-D-ethyldene-glucoside) in advanced neoplastic disease. A phase II study.

Investigators:

Principal: Johannes Blom, M.D.

Objectives: To examine the antitumor effect (remission induction and maintenance) of VP-16-213 in a broad spectrum of metastatic tumors.

Technical Approach: Regimen I: VP-16 60 mg/M<sup>2</sup> twice weekly for four weeks

Regimen II: VP-16 90 mg/M<sup>2</sup> twice weekly for four weeks

Progress & Results: WRAMC entered two patients. One was found dead at home five days after he was entered on the study. The second patient had no response.

ALGB has entered 315 patients, 286 were evaluable at the last analysis in March 1976. Complete and partial response rates with 60 mg and 90 mg regimens were 9% and 12%. A third regimen of 135 mg was added, however, response rate was only 3%. Lymphomas and GI malignancies are still the most responsive tumors.

Conclusion: This drug has some activity in lymphomas and malignancies of the GI tract.

Funding Requirements: See introductory remarks of the Annual Research Report.

Publications: None.

Work Unit No.: 1519

Title of Project: ALGB #7361: Multiple myeloma resistant to 1-phenylalanine mustard treated with cyclophosphamide (cytoxan) (NSC 26271), prednisone (NSC 10023) and 1,3-bis-(2-chloroethyl-1-nitrosourea) (BCNU) (NSC 409962).

Investigators:

Principal: Johannes Blom, M.D.

Objectives: To determine whether patients previously treated with 1-phenylalanine mustard but with recurrent active disease will respond to other alkylating agents (cyclophosphamide and BCNU) and prednisone.

Technical Approach:

Regimen I: cyclophosphamide 600 mg/M<sup>2</sup> IV on day 1  
plus  
prednisone 0.6 mg/kg orally daily for 14 days  
(in 3 equally divided doses  
beginning on day 1)  
0.45 mg/kg orally, daily for 14 days  
0.25 mg/kg orally, daily for 14 days

Then every six weeks:

cyclophosphamide 600 mg/M<sup>2</sup> IV x 1  
plus  
prednisone 0.6 mg/kg/day x 7

Regimen II: cyclophosphamide 300 mg/M<sup>2</sup> IV on day 1  
plus  
BCNU 100 mg/M<sup>2</sup> IV on day 1  
plus  
prednisone 0.6 mg/kg orally daily for 14 days  
(in 3 equally divided doses  
beginning on day 1)  
0.45 mg/kg orally, daily for 14 days  
0.25 mg/kg orally, daily for 14 days

Then every six weeks:

cyclophosphamide 300 mg/M<sup>2</sup> IV x 1  
plus  
prednisone 0.6 mg/kg/day x 7  
plus  
BCNU 100 mg/M<sup>2</sup> IV x 1

**Progress & Results:** WRAMC has entered five patients; two patients had responses, but had progressive disease on day 308 and day 394. Two patients had no response and went off study on day 88 and day 111. The fifth patient had no change, and subsequently expired.

ALGB has entered 62 patients, 52 of whom were evaluable at the March 1976 meeting. Percentages of good response in both regimens was 11% and 20%; limited response 26% and 28%; and no response 63% and 52%. Toxicity was tolerable.

**Conclusion:** Responses with cytoxan and prednisone and BCNU are possible in patients who are resistant to l-phenylalanine mustard.

**Funding Requirements:** See introductory remarks to the Annual Research Report.

**Publications:** None.

Work Unit No.: 1520

Title of Project: ALGB Protocol #7411 - Add. #2: Combination chemotherapy in induction for standard risk and combination chemotherapy plus cranial irradiation plus daunorubicin for increased risk followed by maintenance with continuous vs. intermittent 6-MP plus methotrexate reinforcement and subsequent immunotherapy. Activated 18 April 1974.

Investigators:

Principal: Johannes Blom, M.D.

Associate: Frederick Ruymann, M.D., LTC, MC

Objectives: 1. To assess the role of early cranial radiation in the control of CNS and systemic leukemia by randomly allocating its use.

2. To introduce the concept of more vigorous induction and reinforcement therapy for a group of children considered to be at increased risk; older or younger age (after the 8th birthday or before the 2nd) and/or high leukocyte count (over 30,000), and test whether the addition of daunorubicin will favorably affect the frequency and/or the duration of complete remission in such patients.

3. To compare the effectiveness of three reinforced maintenance regimens:

A. Continuous combined oral 6-MP daily and oral MTX weekly.

B. Intensification with 5-day courses of combined oral MTX weekly.

C. Intensification with 5-day courses of oral MTX alone.

4. To be prepared to introduce immunotherapy in maintenance phase regimens at random.

Technical Approach: Patients are stratified in two risk categories:

Standard Risk: age is after the 2nd and before the 8th birthday and a total white count of less than 30,000.

Increased Risk: age is before the 2nd or after the 8th birthday or the total white blood count is equal to or greater than 30,000.

Patients at standard risk will be allocated to regimens 1 or 2. Patients at increased risk will be allocated to regimens 2 or 3.

Regimen I: vincristine 2.0 mg/ $M^2$ /week IV for 4 weeks on days 1, 8, 15 and 22 plus prednisone 40.0 mg/ $M^2$ /day p.o. for 4 weeks (days 1-28), then taper to 20.0 mg/ $M^2$ /day for 2 days, 10 mg/ $M^2$ /day for 2 days, 5.0 mg/ $M^2$ /day for 2 days, 2.5 mg/ $M^2$ /day for 2 days, then stop prednisone plus methotrexate 12.0 mg/ $M^2$  q 2 weeks IT for six doses on days 1, 15, 22, 43, 50 and 57 plus l-asparaginase 1000 IU/kg/day IV for ten consecutive days from day 29 through 38

Regimen II: vincristine 2.0 mg/ $M^2$ /week IV for 4 weeks on days 1, 8, 15 and 22 plus prednisone 40.0 mg/ $M^2$ /day p.o. for 4 weeks (days 1-28), then taper as Regimen I. plus methotrexate 12.0 mg/ $M^2$  q 2 weeks IT for six doses on days 1, 15, 22, 43, 50 and 57 (last three injections coincide with cranial irradiation) plus l-asparaginase 1000 IU/kg/day IV for ten consecutive days from day 29 through 38 plus cranial irradiation beginning on day 43 (after completion of l-asparaginase) 2400 rads of cranial irradiation over 16 days to day 58.

Regimen III: vincristine 2.0 mg/ $M^2$ /week IV  
for 4 weeks on days 1, 8, 15  
and 22  
plus  
prednisone 40.0 mg/ $M^2$ /day p.o.  
for 4 weeks (days 1-28) and then  
taper as in Regimen I  
plus  
methotrexate 12.0 mg/ $M^2$  q 2 weeks  
IT for six doses on days 1, 15,  
22, 43, 50 and 57 (last three  
injections coincide with cranial  
irradiation)  
plus  
daunorubicin 45.0 mg/ $M^2$ /day IV for  
3 days on days 1, 2 and 3 for  
those 2 years and over and  
22.5 mg/ $M^2$ /day IV for 3 days on  
days 1, 2 and 3 for those under  
2 years of age  
plus  
L-asparaginase 1000 IU/kg/day IV for  
ten consecutive days from day  
29 through 38  
plus  
cranial irradiation beginning on day  
43 (after completion of L-asparagi-  
nase) 2400 rads of cranial irradia-  
tion over 16 days to day 58.

Maintenance phase:

Regimen A: continuous oral 6-MP and MTX:  
6-MP 90.0 mg/ $M^2$ /day orally  
plus  
MTX 15.0 mg/ $M^2$ /week orally on  
the 1st day of each week  
reinforce with vincristine and  
prednisone at monthly intervals  
for five months, thereafter two  
week reinforcement treatments  
are given after the sixth month  
and every three months  
thereafter. The doses are as  
follows:

vincristine 2.0 mg/M<sup>2</sup> IV  
plus  
prednisone 40.0 mg/M<sup>2</sup>/day p.o. for  
one week beginning with the  
vincristine injections -  
(do not taper). When two week  
reinforcements are given,  
prednisone continues for two  
weeks and then is tapered.

Patients induced on regimen 3 with  
daunorubicin will receive  
daunorubicin as part of the  
reinforcement course at the 13th  
and 25th week of maintenance,  
45.0 mg/M<sup>2</sup>/day IV x 2 beginning  
on the 1st day of the vincristine  
plus prednisone reinforcement.

Regimen B: intermittent intensification oral 6-MP  
and oral MTX:  
6-MP 200 mg/M<sup>2</sup>/day orally for five  
days  
plus  
MTX 7.5 mg/M<sup>2</sup>/day orally for five  
days  
wait nine days and then repeat,  
wait nine days and then repeat for  
a third course  
reinforce with vincristine and  
prednisone after every third  
course:  
vincristine 2.0 mg/M<sup>2</sup> IV on days  
1 and 8 for two week reinforce-  
ment treatment  
plus  
prednisone 40.0 mg/M<sup>2</sup>/day p.o. for  
2 weeks and then taper with each  
vincristine reinforcement  
Patients induced on regimen 3 with  
daunorubicin should receive dauno-  
rubicin as part of the reinforcement  
course at the 15th and 31st weeks of  
maintenance, 45.0 mg/M<sup>2</sup>/day IV x 2  
beginning on the first day of  
vincristine and prednisone reinforce-  
ment

Regimen C: intermittent intensification oral  
MTX alone:  
MTX 15.0 mg/M<sup>2</sup>/day orally for five days  
wait nine days and repeat,  
wait nine days and repeat for a third course  
reinforce with vincristine and prednisone after every third course:  
vincristine 2.0 mg/M<sup>2</sup> IV on days 1 and 8 for two week reinforcement treatment  
plus  
prednisone 40.0 mg/M<sup>2</sup>/day p.o.  
for two weeks and tapered,  
with each vincristine reinforcement  
Patients induced on regimen 3 with daunorubicin should receive daunorubicin as part of the reinforcement course at the 15th and 31st weeks of maintenance, 45.0 mg/M<sup>2</sup>/day IV x 2 beginning on the first day of vincristine plus prednisone reinforcement.

**Progress & Results:** WRAMC entered 15 patients. One patient was invalidated because review of the material was more in favor of AML rather than ALL. Eight patients had a complete remission, one of whom relapsed on day 56. The remaining 7 are still in remission from day 239 to day 526. One patient had a partial remission, and then moved to another area where he is being followed by another member of the ALGB. Three patients had progressive disease. Two patients are too early for evaluation, and on two patients no recent information is available.

ALGB has entered 339 patients, 270 of whom were evaluable in March 1976. The marrow remissions continue to be good in all four treatment regimens, varying from 83% to 98%. It is too early to comment upon the incidence of central nervous system relapses. Several patients have developed osteoporosis and fractures, etiology of which is unknown at present. The study continues.

**Conclusion:** The four induction arms have equal remission induction rates. It is too early for further conclusions.

Funding Requirements: See introductory remarks to the Annual Research Report.

Publications: None.

Work Unit No.: 1521

Title of Project: ALGB Protocol #7261 - Add. #1: Comparison of combined triple alkylating agents (L-phenylalanine mustard - NSC 8806, cyclophosphamide - NSC 26271, BCNU (1,3,-bis-(2-chloroethyl)-1-nitrosourea - NSC 409962, plus prednisone with single alkylating agent (L-phenylalanine mustard), plus prednisone for multiple myeloma. A phase III study.

Investigators:

Principal: Johannes Blom, M.D.

Objectives: Comparison of three drugs to the ALGB standard regimen of L-phenylalanine mustard plus prednisone daily to determine whether there is a better response and longer survival.

Technical Approach: Regimen I: L-PAM 0.15 mg/kg/day for seven days by mouth subsequently 0.05 mg/kg/day plus prednisone 1.2 mg/kg/day for 14 days p.o. 0.8 mg/kg/day for 14 days p.o. 0.4 mg/kg/day for 14 days p.o. 0.2 mg/kg/day for 28 days p.o.

Regimen II: BCNU 100 mg/M<sup>2</sup> one dose IV every six weeks plus cytoxan 300 mg/M<sup>2</sup> one dose IV every six weeks plus prednisone - same schedule as above

Progress & Results: WRAMC entered three patients. One was not evaluable and expired on day 160 elsewhere; the second patient had a response but relapsed on day 416; the third patient was invalid because he was entered on the study after the protocol was closed for entry of patients on the 28th of June 1974. Therefore, this constitutes the final report.

Conclusions: Responses are higher in combination treatment than in the L-PAM treatment. L-PAM is less effective with a greater tumor mass than with a smaller one, while the combination treatment appears to be equally effective in both situations.

Funding Requirements: See introductory remarks to Annual Research Report.

Publications: None.

Work Unit No.: 1522

Title of Project: ALGB Protocol #7283 - Add. #2: Treatment of small cell carcinoma of the lung: 1. combination chemotherapy plus radiation therapy vs. 2. single-agent chemotherapy plus radiation therapy with and without prophylactic whole brain radiation. A comparison of cyclophosphamide (NSC 2627), vincristine (NSC 67574), and methotrexate (NSC 740) vs. cyclophosphamide. A phase III study.

Investigators:

Principal: Johannes Blom, M.D.

Objectives: 1. To determine whether the combination chemotherapy (cyclophosphamide, vincristine and methotrexate) plus radiation to the site of the primary disease is more effective in remission induction and prolongation of survival than a single agent (cyclophosphamide) plus radiation in small cell carcinoma of the lung.

2. To determine whether a course of prophylactic whole brain radiation will prevent the onset of cerebral metastasis in this group of patients known to have frequent cerebral metastasis.

3. To establish in a large series the instance of bone marrow dissemination of this tumor at the time of the initial diagnosis.

4. To determine the incidence of ectopic hormone secretion by tumors of the small cell type.

Technical Approach: Induction Phase

Regimen I: cyclophosphamide 2000 mg/M<sup>2</sup> IV on day 1 of the first two 28-day cycles, followed by 1500 mg/M<sup>2</sup> on day 1 of each of the remaining four cycles plus vincristine 1.5 mg/M<sup>2</sup> on day 1 of every 28-day cycle plus methotrexate 30 mg/M<sup>2</sup> IV on day 21 of every 28-day cycle.

Regimen II: cyclophosphamide 2000 mg/M<sup>2</sup> on day 1 of the first two 28-day cycles, followed by 1500 mg/M<sup>2</sup> on day 1 of each of the remaining four cycles

After the second course of chemotherapy patients are randomized between radiation therapy to the tumor and cranium and radiation therapy to the tumor only. Patients who present with CNS involvement were later included in the protocol. Those patients are treated with radiation to the tumor and the brain initially and subsequently with chemotherapy; per addendum #2 on 20 Feb 74 the treatment regimens were changed as follows:

Regimen I: cyclophosphamide and vincristine as before, methotrexate 250 mg/M<sup>2</sup> IV over 12 hrs on day 21 of each cycle plus citrovorum factor to be given by IV push every 6 hrs for six doses.

Regimen II: cyclophosphamide as before plus methotrexate 30 mg/M<sup>2</sup> IV on day 21 of every 28-day cycle.

**Progress & Results:** WRAMC entered 35 patients. Three patients were invalidated for the study. Nine patients had a complete remission, six of whom relapsed from 121 to 500 days. One patient died in complete remission on day 134, and three patients died in the early phase of treatment.

ALGB entered 251 patients, 216 of whom were evaluable in March 1976. Complete plus partial remission rate for the four regimens varied from 30% to 43%, and was not significantly different. Response rates for patients with tumor confined to the lung and mediastinum varied from 30% to 70%, and in this group, the response rate was superior with cyclophosphamide alone. The reasons for this are not apparent. There were no significant differences in toxicity among the various arms. Survival for all patients is equivalent, whether or not maintenance treatment was employed. Although CNS radiotherapy prevents the development of CNS

manifestations, it does not seem to influence the survival time. This study was closed to patient entry on 20 March 1976. This constitutes the final report.

**Conclusions:** Response rates in all treatment regimens are fairly equal. There is no evidence that CNS radiation therapy increases survival.

**Funding Requirements:** See introductory remarks to Annual Research Report.

**Publications:** Proceedings of the 65th Annual Meeting of the American Association for Cancer Research. March 27-30, 1974, page 125, abstract 500.

Work Unit No.: 1523

Title of Project: ALGB Protocol #7421 - Add. #2: Ara-C + Daunorubicin Induction and Ara-C Monthly in Sequential Combination with Thioguanine, Cyclophosphamide, CCNU and Daunorubicin with or without Poly I:C for Maintenance Therapy of AML.

Investigators:

Principal: Johannes Blom, M.D.

Objectives: 1. To determine if a high dose intensive therapy schedule of Ara-C for seven days and Daunorubicin for three days induces a higher incidence and/or longer duration of remission than the same drugs given for five days and two days, respectively, in the therapy of AML.

2. To examine if the therapeutic effect of Ara-C 100 mg/M<sup>2</sup>/24 hours given continuously by IV infusion is different from the same dose given every 12 hours by IV rapid injection in the remission induction therapy of AML.

3. To examine the effect of a sequential administration of Thioguanine, Cyclophosphamide, CCNU and DNR each in combination with a 5-day course of Ara-C given every four weeks, either intravenously or subcutaneously upon the duration of remission. All the drugs mentioned are known to increase the remission duration as compared to unmaintained remissions in past ALGB experience.

4. To determine if the effect of Ara-C given by subcutaneous route is different from that of Ara-C given by the IV route during maintenance therapy.

5. To determine if a synthetic double stranded RNA, Poly I:C, which is an interferon inducer, when added to maintenance chemotherapy produces a prolongation of remission as compared to maintenance chemotherapy alone.

Technical Approach: Patients will be stratified below 60 years and 60 years and over. Below 60 years patients are randomized between four regimens. Above 60 years patients are randomized between the first two regimens only.

Regimen I: Ara-C 100 mg/M<sup>2</sup>/day by continuous IV infusion from day 1 through 5  
plus

Daunorubicin 45 mg/M<sup>2</sup>/day by rapid IV injection on day 1 and 2

Regimen II: Ara-C 200 mg/M<sup>2</sup>/day by rapid IV injection from day 1 through 5  
plus

Daunorubicin 45 mg/M<sup>2</sup> by rapid IV injection on day 1 and 2

Regimen III: Ara-C 100 mg/M<sup>2</sup>/day by continuous IV infusion from day 1 through 7  
plus

Daunorubicin 45 mg/M<sup>2</sup>/day by rapid IV injection from day 1 through 3

Regimen IV: Ara-C 200 mg/M<sup>2</sup>/day by rapid IV injection from day 1 through 7  
plus

Daunorubicin 45 mg/M<sup>2</sup>/day by rapid IV injection from day 1 through 3

Because of better responses with the three and seven day treatments, both above and below the age of 60, regimen I and regimen II were discontinued and all patients are now being treated with three and seven day intravenous doses.

Patients who obtain remission will be randomized between three maintenance regimens. Treatment courses are repeated every 28 days.

Regimen A: Course 1 - Ara-C 200 mg/M<sup>2</sup>/day, day 1-5  
plus  
Thioguanine 200 mg/M<sup>2</sup>/day  
p.o. day 1-5

Course 2 - Ara-C 200 mg/M<sup>2</sup>/day by rapid IV injection, day 1-5  
plus  
Cyclophosphamide 1000 mg/M<sup>2</sup> by rapid IV on day 1

Course 3 - Ara-C 200 mg/M<sup>2</sup>/day by rapid IV injection, day 1-5  
plus  
CCNU 75 mg/M<sup>2</sup> p.o. on day 1 only

Course 4 - Ara-C 200 mg/M<sup>2</sup>/day by  
rapid IV, day 1-5  
plus  
Daunorubicin 45 mg/M<sup>2</sup>/day  
by rapid IV injection on  
day 1 and 2

Regimen B: Identical to Regimen A except Ara-C  
is administered subcutaneously instead  
of intravenously.

Regimen C: Identical to Regimen A but in addition  
Poly I:C is given in the second course  
in the first cycle only.

Progress & Results: WRAMC entered ten patients. One died on day 12 from a subdural hematoma, and a second patient died from sepsis on day 20. Eight patients obtained a complete remission, four of these eight are still in complete remission from 359 to 584 days. One patient was removed from maintenance at another hospital. Three patients relapsed from 91 to 260 days.

ALGB entered 384 patients, 338 of whom were evaluable in March 1976. The complete plus partial response rates for the 5 and 2 regimens was 57% and 49%, and for the 7 and 3 regimens 70% and 62%. The complete response rates for these four regimens are 46%, 38%, 57% and 51%, respectively. The complete response rate for patients 60 years and older in the 5 and 2 regimens is 23% and 14%, and in the 7 and 3 regimens 45% and 37%. The complete plus partial remissions are only slightly higher, 29%, 29%, 55% and 42%, respectively. These responses are higher than compared to previous treatment regimens. Also, a number of patients who failed to respond to the first course of treatment will respond to the second one. In patients 60 years and older treated by the 5 and 2 schedule, there was no significant difference in complications, dependent on the method of Ara-C administration - bolus vs. infusion. Among patients less than 60 years old, those treated with the 7 and 3 schedule had significantly longer survivals than those treated with the 5 and 2 schedule. For this reason, this schedule was discontinued as mentioned under technical approach. The results are suggestive that induction therapy with

continuous infusion with Ara-C is superior to that of bolus administration of Ara-C plus Daunorubicin. The maintenance chemotherapy with subcutaneous Ara-C is significantly superior to that with the intravenous administration of the drug ( $P < 0.01$ ). The apparent detrimental effect of Poly I:C on remission duration must be viewed as inconclusive, pending data review. This study was closed to entry on 15 May 1975. This constitutes the final report.

**Conclusions:** Results with these treatment programs tend to be better than obtained with other treatments so far. Also, the poorer risk patient group: patients 60 years and older, respond better to the 7 and 3 regimen than the 5 and 2 regimen. Duration of remission may be shorter in patients treated with Poly I:C, however, these data are presently being reviewed.

**Funding Requirements:** See introductory remarks to the Annual Research Report.

**Publications:** 11th Annual Meeting of the American Society of Clinical Oncology, May 7-11, 1975, Abstract 1176, page 265.

Work Unit No.: 1524

Title of Project: ALGB Protocol #7251 - Add. #5: A study of 4-drug combination chemotherapy (Vinca Alkaloids and Alkylating Agents) in remission induction and maintenance therapies of stage III & IV Hodgkin's disease.

Investigators:

Principal: Johannes Blom, M.D.

Objectives: 1. To demonstrate the effectiveness of combinations of highly active drugs in prolonged control and potential cure of stage III and IV Hodgkin's disease.

2. To evaluate the role of the individual agents in the 4-drug combinations as a means of selecting the best agents for the control of Hodgkin's disease.

3. To compare the value of VLB maintenance with VLB maintenance with reinforcement courses of each of the 4-drug programs as a means of prolonging control or achieving cure of Hodgkin's disease.

4. To explore the evidence that early splenectomy is beneficial to the patient receiving intensive chemotherapy for his disease.

Technical Approach:

Regimen I: vincristine 1.4 mg/M<sup>2</sup> IV days 1 & 8  
plus  
nitrogen mustard 6 mg/M<sup>2</sup> IV on days 1 & 8  
plus  
prednisone 40 mg/M<sup>2</sup> p.o. daily for 14 days (1st and 4th courses only)  
plus  
procarbazine 100 mg/M<sup>2</sup> p.o. daily for 14 days

Regimen II: vinblastine 4 mg/M<sup>2</sup> IV on days 1 & 8  
plus  
nitrogen mustard 6 mg/M<sup>2</sup> IV on days 1 & 8  
plus  
prednisone 100 mg/M<sup>2</sup> p.o. daily for 14 days (1st and 4th courses only)

Regimen III: vincristine 4 mg/M<sup>2</sup> IV on days 1 & 8  
plus  
CCNU 75 mg/M<sup>2</sup> p.o. day 1  
plus

prednisone 40 mg/M<sup>2</sup> p.o. daily  
for 14 days (1st and 4th courses only)  
plus  
procarbazine 100 mg/M<sup>2</sup> p.o. daily  
for 14 days

Regimen IV: vinblastine 4 mg/M<sup>2</sup> IV on days  
1 & 8  
plus  
CCNU 75 mg/M<sup>2</sup> p.o. on day 1  
plus  
prednisone 40 mg/M<sup>2</sup> p.o. daily  
for 14 days (1st & 4th courses only)  
plus  
procarbazine 100 mg/M<sup>2</sup> p.o.  
daily for 14 days

Each course to be repeated every 28 days for a total  
of six courses.

Maintenance:

Regimen I: vinblastine 6 mg/M<sup>2</sup> IV weekly  
for 8 weeks, thereafter every  
other week

Regimen II: vinblastine 6 mg/M<sup>2</sup> IV weekly  
plus  
reinforcement with a 2 week  
course of the induction regimen  
every 8 weeks

Progress & Results: WRAMC entered 19 patients. Five were invalid or disqualifying. One was transferred to Philadelphia Naval Hospital for further followup. One died from sepsis on day 25. One patient with a partial remission did not return, and was lost to followup. A second patient with a partial remission had a relapse on day 214. One patient had no response. Nine patients had a complete remission, six of whom are still in complete remission from 524 to 1318 days. Three patients with complete remission have relapsed from 260 to 1312 days.

ALGB entered 565 patients, 460 of whom were evaluable in March 1976. Complete remission in the four treatment regimens varied from 63% to 69%, and complete plus partial remissions from 85% to 89%. Although the induction response rates are similar for all four treatment regimens, the treatment with CCNU, vinblastine, procarbazine and

prednisone was superior to the other treatment arms in respect to the duration of response. Twenty-five patients have received the immunotherapy with MER. It is too early to assess the effect of this treatment. This protocol was closed to patient entry on 1 August 1975. This constitutes the final report.

Conclusions: All four treatment regimens appear to be very effective, and equally effective. Earlier results seemed to indicate that remission duration is the longest in the group that was induced with CCNU, vinblastine, procarbazine and prednisone.

Funding Requirements: See introductory remarks to the Annual Research Report.

Publications: Proceedings of the American Society of Hematology, 18th Annual Meeting. December 6-9, 1975, page 60, Abstract 41.

Work Unit No.: 1528

Title of Project: ALGB Protocol #7391 - Add. #0: Clinical trial of radiotherapy and chemotherapy [Cyclophosphamide (NSC 26271), vincristine (NSC 67574) and actinomycin-D (NSC 3053)] in managing non-metastatic Ewing's sarcoma.

Investigators:

Principal: Johannes Blom, M.D.

Associate: Frederick B. Ruymann, M.D., LTC, MC

Objectives: 1. Compare the time interval from clinically localized tumor to appearance of metastases using:

irradiation of the primary tumor only

irradiation of the primary tumor plus systemic chemotherapy (cyclophosphamide, vincristine and dactinomycin)

2. Compare the time interval from clinically localized tumor to appearance of metastases using:

localized irradiation of the primary tumor plus chemotherapy as in 2.12

irradiation of the primary tumor plus chemotherapy as in 2.12 plus bilateral pulmonary irradiation

3. Document the incidence and time of appearance of local recurrence in all patients included in the protocol regimens.

4. Document the total survival time of patients treated by all protocol regimens.

5. Document and evaluate the pattern of organ metastases for all protocol patients who develop metastases so future studies will result in programming improved means of therapy.

Technical Approach: Initial Plan:

Regimen I - vincristine 15 mg/M<sup>2</sup>/week IV x 6

plus

cyclophosphamide 500 mg/M<sup>2</sup>/week IV x 6

plus

radiotherapy to the lesion

Regimen II - vincristine 1.5 mg/ $M^2$ /week x 6

plus

cyclophosphamide 500 mg/ $M^2$ /week IV x 6

plus

radiotherapy to the lesion and both lung fields

Continuation Plan:

actinomycin-D 15 mcg/kg/IV daily x 5 at 3 months after one week's rest vincristine and prednisone are given from the third through the seventh week. These 7-week courses are repeated every 3 months for a total of 6 in 18 months.

Progress & Results: WRAMC entered three patients. One relapsed on day 582. One went off study shortly after entry, and a third patient relapsed. Most recent analysis is of March 1976. Six of 45 patients treated with actinomycin-D, vincristine, cytoxan plus adriamycin relapsed, eighteen of 61 patients who were treated with actinomycin-D, vincristine and cytoxan relapsed, and ten of 55 patients who were treated with actinomycin-D, vincristine, cytoxan plus pulmonary irradiation. Two of 45 patients with actinomycin-D, vincristine, cytoxan plus adriamycin have expired; 4 of 61 who were treated with actinomycin-D, vincristine, and 4 of 65 who were treated with actinomycin-D, vincristine, cytoxan plus pulmonary irradiation.

Conclusion: as above.

Funding Requirements: See introductory remarks to Annual Research Report.

Publications: None.

Work Unit No.: 1529

Title of Project: ALGB Protocol #7253 - Add. #4: Combination chemotherapy of stage III & IV lymphosarcoma and reticulum cell sarcoma with vincristine (NSC 67574), prednisone (NSC 10023), bleomycin (NSC 125066) and streptonigrin (NSC 45383) induction and cyclophosphamide (NSC 26271) maintenance with or without consolidation with vinblastine (NSC 49842), CCNU (NSC 79037) and prednisone (NSC 10023).

Investigators:

Principal: Johannes Blom, M.D.

- Objectives:
1. To ascertain whether the addition of streptonigrin and/or bleomycin to vincristine and prednisone will improve the frequency of complete and partial remissions in patients with lymphosarcoma and reticulum cell sarcoma.
  2. To determine whether consolidation treatment with 2-week courses of vinblastine, prednisone, and CCNU for 16 weeks (four courses with CCNU in 1st and 3rd courses only) after remission is achieved and before maintenance therapy begins will prolong the duration of remissions.
  3. To determine the influence of nodal and extranodal tumor sites upon the course of lymphoreticular disease.

Technical Approach: Regimen I: Vincristine 1 mg/M<sup>2</sup> IV on days 1, 8, 15, 22, 29 and 36  
plus  
prednisone 40 mg/M<sup>2</sup> p.o. daily x 42 days

Regimen II: vincristine 1 mg/M<sup>2</sup> on days 1, 8, 15, 22, 29 and 36  
plus  
prednisone 40 mg/M<sup>2</sup> p.o. daily x 42 days  
plus  
bleomycin 20 mg/M<sup>2</sup> IV on days 1, 8, 15, 22, 29 and 36

Regimen III: vincristine 1 mg/M<sup>2</sup> IV on days 1, 8, 15, 22, 29 and 36  
plus  
prednisone 40 mg/M<sup>2</sup> p.o. daily x 42 days  
plus  
streptonigrin 1 mg/M<sup>2</sup> p.o. spaced over 1 hr, days 1, 8, 15, 22, 29 and 36

Regimen IV: vincristine 1 mg/M<sup>2</sup> IV on days 1, 8, 15, 22, 29 and 36  
plus  
prednisone 40 mg/M<sup>2</sup> daily x 42 days  
plus  
bleomycin 20 mg/M<sup>2</sup> IV on days 1, 8, 15, 22, 29 and 36  
plus  
streptonigrin 1 mg/M<sup>2</sup> p.o. spaced over 1 hr, days 1, 8, 15, 22, 29 and 36

Patients who obtain a complete or partial remission are randomized between maintenance with consolidations and maintenance only.

Consolidations: vinblastine 4 mg/M<sup>2</sup> days 1 & 8  
plus  
prednisone 40 mg/M<sup>2</sup> p.o. x 14 days  
plus  
CCNU 80 mg/M<sup>2</sup> p.o. on day 1

Repeat this course every 28 days for four courses.

Maintenance: cyclophosphamide 2 mg/kg/day p.o.

Progress & Results: WRAMC entered four patients. One had a partial remission and relapsed on day 40, and subsequently expired. Two patients obtained a complete remission one of whom is still in remission at 916 days, and the second patient relapsed on day 686. The fourth patient is presently in a good partial remission, and continues on study.

ALGB has entered 520 patients, 443 of whom were evaluable in March 1976. Complete responses in lymphosarcoma vary from 42% to 57% and complete plus partial responses from 83% to 86%. There is no significant difference between any of the four induction regimens. For reticulum cell sarcoma, these figures are complete remissions from 31%

to 50% and complete plus partial remissions from 70% to 87%. Also here there is no significant difference yet. The duration of responses is consistently lower in patients with histiocytic lymphoma than in those with lymphocytic lymphomas. At the present time, there are no significant differences in survival based upon induction treatment or the presence or absence of extranodal disease. This study was closed to entry on 20 January 1976. This constitutes the final report.

**Conclusions:** There is no significant difference in response rate between the four treatment regimens in lymphosarcoma and reticulum cell sarcoma.

**Funding Requirements:** See introductory remarks to the Annual Research Report.

**Publications:** None.

Work Unit No.: 1530

Title of Project: ALGB Protocol #7132 - Add. #3: Prevention of blast crisis in chronic myelocytic leukemia by the use of pulsed doses of 1,(2-chloroethyl)3-cyclohexyl-1-nitrosourea (CCNU, NSC 79037).

Investigators:

Principal: Johannes Blom, M.D.

Objectives: To determine whether pulsed doses of CCNU and Ara-C during long term maintenance of remission in CML with Busulfan will prevent or postpone the development of myeloblastic crisis.

Technical Approach: Induction: Busulfan 4.0 mg/M<sup>2</sup> daily

Maintenance: Regimen I: Busulfan in a dose to maintain the WBC in between 5000 and 15000

Regimen II: Busulfan in a dose to maintain the WBC in between 5000 and 15000 for 5 weeks to be followed by CCNU 75 mg/M<sup>2</sup> p.o. plus Ara-C 100 mg/M<sup>2</sup> SC x 2 in 24 hrs.

Four of these courses will be given yearly.

Progress & Results: WRAMC entered five patients. One relapsed on day 1125, and another patient relapsed on day 512. Three patients are still on study from 643 to 984 days.

ALGB entered 251 patients, 224 of whom were evaluable at the March meeting in 1976. Response rate and survival are similar for both regimens, and although the experimental arm with CCNU and Ara-C does not prevent the development of a blastic crisis, it is at least as effective as the Busulfan alone in the control of chronic myelocytic leukemia. The study was closed to patient entry on 20 August 1975. This constitutes the final report.

Conclusions: Although the experimental arm does not prevent the development of blastic crisis, it is at least as effective in the control of chronic myelocytic leukemia as the conventional Busulfan.

Funding Requirements: See introductory remarks to the Annual Research Report.

Publications: None.

Work Unit No.: 1531

Title of Project: ALGB Protocol #7113 - Add. #4: L-asparaginase/vincristine/daunorubicin induction of ALL in adults followed by comparison of daily 6-MP, weekly methotrexate and monthly vincristine/prednisone reinforcement doses with high dose intensive courses of parenteral methotrexate and 6-MP.

Investigators:

Principal: Johannes Blom, M.D.

- Objectives:
1. This protocol will include a treatment with L-asparaginase during induction in an attempt to increase the yield of complete remissions in adult ALL.
  2. The main thrust of this protocol will be to assess the value in extending complete remissions of intensive combination chemotherapy with methotrexate and 6-MP given either as paired sequential high-dose courses or as continuous incremental administration with periodic reinforcement therapy using vincristine and prednisone.
  3. Prophylaxis against meningeal leukemia will consist of intrathecal methotrexate plus cranial irradiation. The influence of intrathecal methotrexate upon the probability of achieving marrow remission will be assessed.

Technical Approach: Induction: vincristine 2.0 mg IV on days 1, 8, 15 plus prednisone  $40.0 \text{ mg/M}^2/\text{day}$  p.o. until M-1 marrow or until day 50 plus prednisone  $20.0 \text{ mg/M}^2/\text{day}$  for 2 days  $10.0 \text{ mg/M}^2/\text{day}$  for 2 days  $5.0 \text{ mg/M}^2/\text{day}$  for 2 days  $2.5 \text{ mg/M}^2/\text{day}$  for 2 days and then discontinue. plus L-asparaginase 500 IU/kg/day IV for 10 days beginning on day 16 with or without methotrexate  $12.0 \text{ mg/M}^2/\text{week}$  intrathecally on days 1, 8, 15 If no M-1 is obtained by day 29 daunorubicin  $45.0 \text{ mg/M}^2$  IV weekly up to 4 doses on days 29, 36, 43 and 50 or until M-1 marrow is reached.

Intensification: Regimen I: MTX 15.0 mg/M<sup>2</sup>/day IM x 5  
repeat IT after at least  
9 day rest period  
plus  
6-MP 600 mg/M<sup>2</sup>/day IV x 5  
repeat IT after at least  
9 day rest period

Regimen II: MTX 15.0 mg/M<sup>2</sup> weekly p.o.  
plus  
6-MP 90.0 mg/M<sup>2</sup> daily p.o.

After this cranial radiation 2400 rads  
x 2 weeks.

After this continuation of regimen I or  
regimen II for a total of 1 year with  
vincristine and prednisone reinductions.

Progress & Results: WRAMC entered six patients. One was disqualified because the diagnosis was AML rather than ALL. The second patient obtained a partial remission and died from pneumocystis pneumonia on day 65. One patient had a partial remission and relapsed on day 104. Two patients obtained a complete remission one of whom has been in remission for 170+ days, the second one is in the early phase of maintenance. One patient is in the early stages of treatment.

ALGB entered 124 patients, 112 of whom were evaluable in March 1976. The induction remission rate continues to be approximately 75%, 25% of the patients remain in complete remission. There appears to be no difference among the maintenance arms. This study will be closed as soon as the new study is activated. This, therefore, constitutes the final report.

Conclusions: There is no difference between the induction arm, approximately 75% remission rate, with approximately 25% patients who remain in complete remission.

Funding Requirements: See introductory remarks to the Annual Research Report.

Publications: None.

Work Unit No.: 1532

Title of Project: ALGB Protocol #7451 - Add. #0: Combination radiotherapy and chemotherapy of stage III Hodgkin's disease. (Phase III)

Investigators:

Principal: Johannes Blom, M.D.

Associate: Henry Keys, MAJ, MC, USA

Objectives: 1. To compare long-term, multiple-agent chemotherapy either alone or in combination with total nodal radiotherapy with total nodal radiation therapy alone.

2. To compare tolerance of patients to these treatments of various intensities.

3. To compare the quality of response, duration of response and survival rates of the therapeutic groups.

4. To compare tolerance of therapy for patients with and without prior splenectomy for staging.

5. To study patterns of relapse in the various study groups.

Technical Approach: Regimen I: total nodal radiation therapy with the mantle port above the diaphragm and inverted "Y" below the diaphragm plus the spleen or splenic pedicle area and optionally the porta hepatis.

Regimen II: chemotherapy consisting of:  
vincristine 1.4 mg/M<sup>2</sup>/week IV x 2 with  
a maximum dose of 2.0 mg  
plus  
procarbazine 50.0 mg on day 1 p.o.  
100.0 mg on day 2 p.o.  
100.0 mg/M<sup>2</sup>/day on days  
3-14 p.o.  
plus  
BCNU 80.0 mg/M<sup>2</sup> IV on day 1

Each course will consist of 2-weeks treatment and 2-weeks rest.

The 2nd and 3rd course will be as described above with the deletion of prednisone.

The 4th course is the same as the 1st course with prednisone included.

The 5th and 6th course is the same as the 2nd and 3rd course - vincristine/procarbazine/BCNU with the prednisone.

Maintenance therapy will be given for 3 years consisting of:

chlorambucil 6.0 mg/ $M^2$ /day p.o.

Regimen III: Chemotherapy followed by radiation therapy.

Six cycles of chemotherapy as outlined under regimen II will be followed by a 2-month rest period and then total nodal radiation as described under regimen I.

No maintenance drugs will be given.

**Progress & Results:** WRAMC has entered two patients, both of whom obtained a complete remission; one relapsed on day 203, and the second one is still in remission on day 312.

ALGB has entered 45 patients, 39 of whom were evaluable in March 1976. It is too early for any conclusions.

**Conclusions:** Too early.

**Funding Requirements:** See introductory remarks to the Annual Research Report.

**Publications:** None.

Work Unit No.: 1533

Title of Project: ALGB Protocol #7461 - Add. #1: Primary treatment of multiple myeloma: comparison of L-PAM (NSC 8806) plus prednisone (NSC 10023) and BCNU (NSC 409962) plus prednisone and CCNU (NSC 79037) plus prednisone with or without intermittent vincristine (NSC 67574) and prednisone. A phase III study.

Investigators:

Principal: Johannes Blom, M.D.

Objectives: 1. To compare the relative response inducing capabilities of CCNU plus prednisone, BCNU plus prednisone, and L-PAM plus prednisone in multiple myeloma.

2. To study the effectiveness of intermittent reinforcement doses of vincristine and prednisone added to the therapies described under 1 in multiple myeloma.

Technical Approach: Regimen I: L-PAM 150 mcg/kg/day x 7 p.o.  
plus  
prednisone .8 mg/kg/day x 14 p.o.  
beginning on day 1  
.4 mg/kg/day x 14 p.o.  
.2 mg/kg/day x 14 p.o.  
  
3-4 weeks after the loading dose of L-PAM when the peripheral counts are rising daily maintenance with L-PAM will be started in a dose of 50.0 mcg/kg/day p.o.

Regimen II: BCNU 150 mg/M<sup>2</sup> IV every 6 weeks  
plus  
prednisone as described under regimen I.

Regimen III: CCNU 100 mg/M<sup>2</sup> p.o. every 6 weeks  
plus  
prednisone as described under regimen I.

On day 154 (at the end of week 22) all patients who have not shown relapse or progressive disease will be randomized again.

Regimen A indicates that the patient should continue with initial therapy and receive no additional therapy.

Regimen B indicates that the patient should continue with his initial therapy and in addition receive vincristine 1.0 mg/M<sup>2</sup> IV x 1 on day 154 and every 8 weeks thereafter plus prednisone 0.6 mg/kg/day p.o. x 7 beginning on day 154 and every 8 weeks thereafter.

During maintenance phase the interval between doses of BCNU or CCNU is increased from 6 to 8 weeks.

Addendum #1 dated 24 January 1975 adds a

Regimen IV: L-PAM 16.0 mg/M<sup>2</sup> IV every 2 weeks for 6 weeks and then every 4 weeks plus prednisone as outlined under Regimen I.

Progress & Results: WRAMC entered four patients. One had progressive disease, one had improvement of the serum proteins but developed extensive myopathy and subsequently expired, one patient obtained a good remission but relapsed on day 593, the fourth patient was recently entered.

ALGB entered 247 patients, 210 of whom were evaluable in March 1976. There has been no significant difference in response rates thus far, with 60% of patients achieving good or limited responses.

Conclusions: No significant difference in response rate thus far.

Funding Requirements: See introductory remarks to Annual Research Report.

Publications: None.

Work Unit No.: 1534

Title of Project: ALGB Protocol #7521. Add. #2: A comparative study of the value of immunotherapy with MER as adjuvant to induction and two maintenance chemotherapy programs in acute myelocytic leukemia. A Phase III Study.

Investigators:

Principal: Johannes Blom, M.D.

- Objectives:
1. To determine whether early immunotherapy with MER in conjunction with a primary chemotherapeutic induction program will increase the probability of achieving complete remission.
  2. To compare remission duration and survival with respect to two types of maintenance chemotherapy, one using monthly courses of Ara-C and 6-thioguanine, the other using alternating monthly courses of Ara-C and thioguanine with vincristine, dexamethasone and Ara-C.
  3. To determine by concurrent comparative controlled trial if MER immunotherapy will prolong remission duration and increase the survival time of patients with AML receiving either of two plans of concomitant chemotherapy.
  4. To determine if the frequency of CNS leukemia and of toxicity to chemotherapy is different in patients randomly assigned to receive maintenance chemotherapy with or without vincristine and dexamethasone and with or without MER.
  5. In two programs of maintenance chemotherapy, to assess the morbidity and toxicity of MER immunotherapy.

Technical Approach: Induction Regimen is the same for all patients, consisting of:

cytosine arabinoside 100 mg/M<sup>2</sup>/day by continuous infusion from day 1 thru day 7

plus

Daunorubicin 45 mg/M<sup>2</sup>/day by rapid IV injection on days 1, 2 and 3.

If the bone marrow contains more than 5% leukemic cells, patient will receive a second course of cytosine arabinoside, this time for 5 days plus daunorubicin for 2 days.

Patients will be randomized for MER or no MER during the Induction Phase.

The Maintenance Phase consists of:

Regimen A: 5-day courses repeated every 4 weeks, consisting of:

cytosine arabinoside 100 mg/M<sup>2</sup> s.c.  
every 12 hrs for 10 injections  
plus  
thioguanine 100 mg/M<sup>2</sup> p.o. every 12 hrs  
for a total of 10 doses  
plus  
MER

Regimen B: cytosine arabinoside 100 mg/M<sup>2</sup> s.c.  
every 12 hrs for a total of 10  
injections  
plus  
thioguanine 100 mg/M<sup>2</sup> p.o. every 12 hrs  
for a total of 10 doses

Alternate with Second five day course:

cytosine arabinoside 100 mg/M<sup>2</sup> s.c.  
injection every 12 hrs, total of 10  
injections on days 1 thru 5  
plus  
vincristine 2 mg/M<sup>2</sup>, 2 mg max., on  
day 1 of this course  
plus  
dexamethasone 8 mg/M<sup>2</sup>, not to exceed  
16 mg p.o. in 3 divided doses daily  
on day 1 thru 5  
plus  
intradermal MER

Regimen C: 5-day course repeated every 4 weeks  
cytosine arabinoside 100 mg/M<sup>2</sup> s.c.  
every 12 hrs, total of 10 injections  
plus  
thioguanine 100 mg/M<sup>2</sup> p.o. every 12 hrs  
for a total of 10 doses

In all 3 regimens, the 3rd, 7th, 11th and 15th courses are substituted for cytosine arabinoside 100 mg/M<sup>2</sup> s.c. every 12 hours, total of 10 injections  
plus  
daunorubicin 45 mg/M<sup>2</sup>/day by rapid IV injection on days 1 and 2.

Progress & Results: WRAMC has entered twelve patients. Four expired during the induction phase; one patient had no response; two patients are too early; five patients went into complete remission, one relapsed on day 114, one relapsed on day 128, three are still in remission from 4 to 253 days.

ALGB has entered 177 patients, 107 of whom were evaluable in March 1976. No difference is seen yet in the percentage of complete response, whether or not MER was given during induction. It is too early to comment upon remission duration in this study.

Conclusions: Too early for any definite conclusions at the present time.

Funding Requirements: See introductory remarks to Annual Research Report.

Publications: None.

Work Unit No.: 1535

Title of Project: ALGB Protocol #7581 - Add. #1: Long term surgical adjuvant systemic chemotherapy with or without adjuvant immunotherapy in mammary carcinoma. A comparative study of cytoxan, vincristine, methotrexate, 5-fluorouracil, prednisone vs. cytoxan, methotrexate, 5-fluorouracil vs. cytoxan, methotrexate, 5-fluorouracil, MER. A Phase III Study.

Investigators:

Principal: Johannes Blom, M.D.

Objectives: 1. It is the specific aim of this study to ascertain if therapy with 3 active agents plus nonspecific immunostimulation is superior to the 3 active agents given alone, or given in combination with vincristine and prednisone. The criteria for assessment will be the disease free interval of breast cancer patients with 4 or more positive axillary nodes discovered at mastectomy. A corollary comparison to the historical information in a patient group similarly staged and operated when followed by observation alone or by 3 active agent therapy in Milan will be utilized for an additional comparison.

2. The duration of the disease free interval in each treatment will be evaluated for its impact upon survival, as well as serving the principle measure of therapeutic effect.
3. Patient tolerance to the therapeutic regimens will be evaluated.
4. The site of first recurrence of disease will be evaluated to determine any differential action of the regimens.
5. An attempt will be made to determine if patient age, primary lesion size, or the utilization of postoperative radiotherapy influenced the recurrence or survival rates, as well as the location of the site of first recurrence.

Technical Approach: Induction Phase Treatment Schedules

Regimen I: cytoxan  $80 \text{ mg}/\text{M}^2/\text{day}$  orally for 42 consecutive days  
plus  
methotrexate  $40 \text{ mg}/\text{M}^2/\text{week IV}$  for 6 consecutive weeks  
EXCEPT patients 60 years of age are to receive  $30 \text{ mg}/\text{M}^2/\text{week IV}$

plus  
5-FU 500 mg/M<sup>2</sup>/week IV for 6 consecutive weeks  
plus  
vincristine 1.0 mg/M<sup>2</sup>/week IV for 6 consecutive weeks (max. dose 1.5 mg per dose)  
plus  
prednisone 40 mg/M<sup>2</sup>/day orally daily in 3 divided doses for 21 consecutive days followed by half dose for 2 consecutive days; followed by quarter dose for 2 consecutive days; followed by one-eighth dose for 2 days, then discontinue

Treatment will begin no sooner than two weeks and not later than four weeks following mastectomy in those patients not receiving postoperative radiotherapy. If postoperative radiotherapy is given, chemotherapy will begin no sooner than 4 weeks and not later than 8 weeks following completion of radiotherapy (and not later than 16 weeks from mastectomy)

Regimen II: cytoxan 80 mg/M<sup>2</sup>/day orally for 42 consecutive days  
plus  
Methotrexate 40 mg/M<sup>2</sup>/week IV for 6 consecutive weeks, EXCEPT patients 60 years or older are to receive 30 mg/M<sup>2</sup>/week IV  
plus  
5-FU 500 mg/M<sup>2</sup>/week IV for 6 consecutive weeks

Regimen III: cytoxan 80 mg/M<sup>2</sup>/day orally for 42 consecutive days  
plus  
methotrexate 40 mg/M<sup>2</sup>/week IV for 6 consecutive weeks EXCEPT patients 60 years or older are to receive 30 mg/M<sup>2</sup>/week IV  
plus  
5-FU 500 mg/M<sup>2</sup>/week IV for 6 consecutive weeks  
plus  
MER 200 ug intradermally in each of 5 sites (total 1 mg) at weeks 1, 3 and 5

MER should be swirled in the vial and repeatedly tilted in the tuberculin syringe to assure its homogeneous suspension. Injection sites should be chosen to drain into different node groups. Do not inject lymphadematous arm.

Maintenance Phase Treatment Schedules for First Year of Maintenance

Regimen I: cytoxan 100 mg/M<sup>2</sup>/day orally days 1-14 of each cycle  
plus methotrexate 40 mg/M<sup>2</sup> IV day 1 and day 8 of each cycle EXCEPT patients 60 years or older are to receive 30 mg/M<sup>2</sup>  
plus 5-FU 500 mg/M<sup>2</sup> IV day 1 and day 8 of each cycle  
plus vincristine 1.0 mg/M<sup>2</sup> IV day 1 and day 8 of each cycle (max. dose 1.5 mg/dose)  
plus prednisone 40 mg/M<sup>2</sup>/day orally days 1-14 of each cycle DO NOT TAPER

Each cycle of therapy is 28 days in length and recycle begins on day 29. This regimen should be given for 10 cycles, after which patients enter the Second Year of Maintenance (see below)

Regimen II: cytoxan 100 mg/M<sup>2</sup>/day orally days 1-14 of each cycle  
plus methotrexate 40 mg/M<sup>2</sup> IV days 1 and day 8 of each cycle EXCEPT patients 60 years or older are to receive 30 mg/M<sup>2</sup>  
plus 5-FU 500 mg/M<sup>2</sup> IV day 1 and day 8 of each cycle

Each cycle of therapy is 28 days in length and recycle begins on day 29. This regimen should be given for 10 cycles, after which patients enter the Second Year of Maintenance

Regimen III: cytoxan 100 mg/M<sup>2</sup>/day orally days 1-14 of each cycle  
plus  
methotrexate 40 mg/M<sup>2</sup> IV days 1 and day 8 of each cycle EXCEPT patients 60 years or older are to receive 30 mg/M<sup>2</sup>  
plus  
5-FU 500 mg/M<sup>2</sup> IV day 1 and day 8 of each cycle  
plus  
MER 200 ug intradermally in each of 5 sites (total 1 mg) on day 8 of each cycle.

Each cycle of therapy is 28 days in length and recycle begins on day 29. This regimen should be given for 10 cycles, after which patients enter the Second Year of Maintenance.

#### Maintenance Phase Treatment Schedule for Second Year of Maintenance

At the scheduled time for the 11th cycle of maintenance therapy, patients in all 3 regimens will begin a uniform treatment schedule. Vincristine and prednisone are dropped from regimen I; MER is dropped from regimen III and the length of a treatment cycle is increased to 56 days.

In the second year of maintenance, all patients will receive:

cytoxan 100 mg/M<sup>2</sup>/day orally days 1-14 of each cycle  
plus  
methotrexate 40 mg/M<sup>2</sup> IV on day 1 and day 8 of each cycle EXCEPT patients 60 years or older are to receive 30 mg/M<sup>2</sup>  
plus  
5-FU 500 mg/M<sup>2</sup> IV on day 1 and day 8 of each cycle

Each cycle of therapy is 56 days in length and recycle begins on day 57. Treatment should continue for 6 cycles, after which, all treatment is discontinued and the patient should be observed indefinitely at 3 month intervals without further therapy.

**Progress & Results:** WRAMC has entered six patients, all of whom remain in complete remission from 46 to 335 days. Forty-nine patients have been entered, 37 of whom were evaluable in March 1976. Toxicity in all arms has been acceptable. MER toxicity has been tolerable, although several patients have had their treatment interrupted to allow healing of local MER lesions. There has been only one recurrence to date.

**Conclusions:** It is too early for any conclusions.

**Funding Requirements:** See introductory remarks to Annual Research Report.

**Publications:** None.

Work Unit No.: 1536

Title of Project: ALGB Protocol #7531. Add. #0: Treatment of chronic myelocytic leukemia with the aim of the prevention of myeloblastic transformation. A Phase III Study.

Investigators:

Principal: Johannes Blom, M.D.

Objectives: To determine whether longer courses of CCNU and Ara-C, begun at time of diagnosis and without Busulfan, can postpone or prevent myeloblastic transformation.

Technical Approach: Regimen I: busulfan 4 mg/M<sup>2</sup> daily for induction and maintenance

Regimen II: CCNU 35 mg/M<sup>2</sup> orally every 6 weeks plus Ara-C 50 mg/M<sup>2</sup> s.c. q 12 hours on days 1-5 of each 6 week cycle.

Progress & Results: WRAMC has entered one patient, who had progressive disease on day 84.

ALGB has entered thirteen patients, six of whom were evaluable in March 1976.

Conclusions: Too early.

Funding Requirements: See introductory remarks to Annual Research Report.

Publications: None.

Work Unit No.: 1537

Title of Project: ALGB Protocol #7551, Add. #0: Combination chemotherapy and radiotherapy for stage IV Hodgkin's disease, no prior treatment.

Investigators:

Principal: Johannes Blom, M.D.

Objectives: 1. To compare the response rates and remission durations observed with 6 or 12 monthly cycles of chemotherapy.

2. To determine the effectiveness of a combined approach by radiotherapy and multiple drug chemotherapy in the control of Stage IV Hodgkin's Disease as compared to multiple drug chemotherapy alone.

3. To explore whether early reduction of bulk disease by radiotherapy is beneficial in controlling the disease.

4. To explore the ability of radiotherapy to eradicate residual microscopic disease in patients with apparent complete remission after a full course of multiple drug chemotherapy.

5. To explore the ability of radiotherapy to eradicate disease in patients with apparent partial remission after a full course of multiple drug chemotherapy.

Technical Approach: Regimen I: CCNU 75 mg/M<sup>2</sup> p.o. day 1  
vinblastine 4 mg/M<sup>2</sup> IV day 1 and 8  
procarbazine 100 mg/M<sup>2</sup> p.o. day 1  
thru 14  
prednisone 40 mg/M<sup>2</sup> p.o. day 1 thru 14

Prednisone is given on course 1 and 4 only.

After each course of treatment, there is a 2 week rest period. This treatment is given for a total of six courses.

Regimen II: Is the same as Regimen I, but the therapy should continue for a total of twelve courses.

The prednisone is given on courses 1, 4, 7 and 10 only.

Regimen III: Consists of six months of chemotherapy, as outlined in Regimen I, plus radiation therapy.

Regimen IV: Is the same chemotherapy as outlined in Regimen I, to be given for three courses, after which radiation therapy will be administered. Four weeks after the completion of radiation, another three courses of chemotherapy will be administered.

The radiation therapy will consist of 2500 rads to be given in 4 weeks to areas of gross disease known to exist prior to the start of chemotherapy.

All patients will be placed on maintenance therapy which will consist of:

chlorambucil 6 mg/M<sup>2</sup> given daily for a total of 3 years, or until progressive disease.

Progress & Results: WRAMC has entered one patient.

ALGB has entered ten patients, five of whom are evaluable. It is too early for any evaluation.

Conclusions: Too early.

Funding Requirements: See introductory remarks to the Annual Research Report.

Publications: None.

Work Unit No.: 1538

Title of Project: CALGB Protocol #7552. Add. #1: Combination chemotherapy and immunotherapy for previously treated Stage III and IV Hodgkin's Disease.

Investigators:

Principal: Johannes Blom, M.D.

Objectives: 1. To compare remission rates and the remission duration of two, four drug chemotherapy regimens employing completely different agents in previously treated patients with Stage IV Hodgkin's Disease.

2. To compare the response rates and remission durations of the repetitive use of the four drug combination regimens with alternating cycles of the two entirely different regimens, thus exposing the patient to eight drugs.

3. To compare the efficacy of chemotherapy and chemoimmunotherapy with respect to response rates, remission durations, and toxicity.

4. To assess immunological tests of delayed MER hypersensitivity as prognostic indices, and to compare the effects of different combined chemotherapies and of immunotherapy upon them.

Technical Approach: Regimen IA or CCNU 75 mg/M<sup>2</sup> p.o. on day 1  
Regimen IB plus  
vinblastine 4 mg/M<sup>2</sup> IV on days  
1 and 8  
plus  
procarbazine 100 mg/M<sup>2</sup> p.o. on  
days 1 thru 14  
plus  
prednisone 40 mg/M<sup>2</sup> p.o. on days  
1 thru 14

Prednisone is included in courses 1, 4, 7, and 10 only.

Patients randomized to Regimen IA will receive in addition to this chemotherapy, immunotherapy with MER 200 ug intradermally in each of 5 sites, to be administered on the first day of each course.

Patients randomized to Regimen IB will receive chemotherapy only.

Regimen IIA bleomycin 5 units/ $M^2$  I.V. on days 1  
or Regimen IIB and 8  
plus  
adriamycin 50 mg/ $M^2$  I.V. on day 1  
(max. total dose 550 mg/ $M^2$ )  
plus  
vincristine 1.4 mg/ $M^2$  I.V. on days 1  
and 8  
plus  
streptozotocin 1500 mg/ $M^2$  I.V. on  
days 1 and 8

After each 2 week treatment period, there will be a 2 week rest period. Patient will receive a total of 12 courses.

Patients randomized to Regimen IIA will receive in addition to this chemotherapy, immunotherapy with MER, 200 ug intradermally in each of 5 sites, to be administered on the first day of each course.

Patients randomized to Regimen IIB will receive Regimen II chemotherapy only.

Regimen IIIA Will consist of 12 courses of induction or Regimen IIIB therapy. Each course will consist of 2 weeks of chemotherapy, and a course will be given every 4 weeks.

Regimen III will be alternate courses of Regimen I and Regimen II chemotherapy.

Patients randomized to Regimen IIIA will receive in addition to this chemotherapy, immunotherapy with MER, 200 ug intradermally in each of 5 sites, to be administered on the first day of each course.

Patients randomized to Regimen IIIB will receive Regimen III chemotherapy only.

Maintenance Therapy: At the end of 12 courses of induction therapy, all patients who are in complete or partial remission status will receive: chlorambucil 6 mg/ $M^2$ /day

Progress & Results: WRAMC has entered two patients.

CALGB has entered twenty patients, eight of whom are evaluable. It is too early for any results or conclusions.

Conclusions: Too early.

Funding Requirements: See introductory remarks to the Annual Research Report.

Publications: None.

Work Unit No.: 1539

Title of Project: CALGB Protocol #7541. Add. #0: Combination chemotherapy and immunotherapy in previously untreated Stage III and IV neuroblastoma. A Phase III Study.

Investigators:

Principal: Johannes Blom, M.D.

- Objectives:
1. To evaluate the role of triple-drug (vincristine, cyclophosphamide, adriamycin) combination chemotherapy in previously untreated Stage III and IV neuroblastoma
  2. To evaluate the immunological responsiveness of patients with disseminated neuroblastoma, both prior to and during therapy.
  3. To evaluate the role of an agent (MER) thought capable of stimulating immunological responsiveness both in terms of the patient's immunological reactivity (to skin tests) and in terms of possible contribution to prolongation of median survival.

Technical Approach: Regimen I: vincristine 1.5 mg/ $M^2$  I.V. on days 1, 8, 29, 36, 57, 64, 85, 92 and for a similar schedule (two weeks out of every four) for a total of 1 year plus cyclophosphamide 500 mg/ $M^2$  on days 1, 57, and every 2 months thereafter for 1 year, and 1,000 mg/ $M^2$  on days 29, 85, and every 2 months thereafter for 1 year plus adriamycin 25 mg/ $M^2$ /day x 3 I.V. beginning on days 1, 57, and every 2 months thereafter

Regimen II: vincristine 1.5 mg/ $M^2$  I.V. on days 1, 8, 29, 36, 57, 64, 85, 92, and for a similar schedule (two weeks out of every four) for a total of 1 year. plus cyclophosphamide 500 mg/ $M^2$  on days 1, 57, and every 2 months thereafter for 1 year, and 1,000 mg/ $M^2$  on days 29, 85, and every 2 months thereafter for 1 year plus

adriamycin 25 mg/M<sup>2</sup>/day x 3 I.V.  
beginning on days 1, 57, and every  
2 months thereafter,  
plus  
MER 200 ug in each of 5 sites (total  
1 mg) intradermally on days 8, 36,  
64 and every fourth week thereafter.

Treatment Procedure:

1. Laparotomy and tumor resection will be performed as appropriate.
2. Patients with Stage III disease will have scheduled radiotherapy beginning 5 weeks after the 1st course of chemotherapy, proving hematologic thresholds are satisfied.
3. Patients with Stage IV disease will have radiotherapy used electively, beginning 5 weeks after the 1st dose of chemotherapy, providing hematologic thresholds are satisfied, unless emergency indicated appearance beforehand. The radiation therapy will be given in 180-200 rad fractions, at a rate of 1 fraction per day for a total schedule of 5 fractions per week.

Progress & Results: No patients have been entered at WRAMC.

This protocol was activated recently, and also in March 1976 there were no data available of entries by CALGB.

Conclusions: Too early.

Funding Requirements: See introductory remarks to the Annual Research Report.

Publications: None.

Work Unit No.: 1540

Title of Project: CALGB Protocol 7582. Add. #0: Procarbazine, Vinblastine and Dactinomycin in Stage III and IV melanoma with or without MER. (A comparative Phase II Study.)

Investigators:

Principal: Johannes Blom, M.D.

Objectives: 1. To assess the effectiveness of a combination of Procarbazine, Vinblastine and Dactinomycin against metastatic melanoma.

2. To assess the effectiveness of the addition of MER to the above program.

3. To informally assess the effectiveness of radiation therapy in those patients who develop CNS metastasis during the course of treatment with Procarbazine, Vinblastine and Dactinomycin with or without MER.

Technical Approach: Regimen I: vinblastine  $5 \text{ mg}/\text{M}^2$  I.V. on days 1 and 8  
dactinomycin  $0.5 \text{ mg}/\text{M}^2$  I.V. on days 1 and 8  
procarbazine  $100 \text{ mg}/\text{M}^2$  p.o. on days 1 thru 10

This treatment regimen is repeated every 28 days.

Regimen II: The chemotherapy regimen is the same as Regimen I, plus 1 mg of MER intra-dermally on day 1 of each cycle. The 1 mg of MER will be divided in 5 equal doses, and injected at 5 different sites.

Progress & Results: WRAMC has not entered any patients.

CALGB has entered 22 patients, 7 of whom were evaluable in March 1976.

It is too early for any results or conclusions.

Results & Conclusions: Too early.

Funding Requirements: See introductory remarks to the Annual Research Report.

Publications: None

Work Unit No.: 1541

Title of Project: CALGB Protocol #7542. Add. #0: Protocol for the treatment of Non-Hodgkin's lymphomas in children. Methotrexate, Vincristine, Dexamethasone, Cyclophosphamide, 6-Mercaptopurine plus radiation therapy to involved areas. A Phase III Study.

Investigators:

Principal: Johannes Blom, M.D.

Associate: Frederick B. Ruymann, M.D., LTC, MC

Objectives: 1. To develop a combined radiotherapy/chemotherapy regimen which will increase the survival and cure rate in children with non-Hodgkin's lymphoma not previously treated.

2. To determine the efficacy of the addition of daily oral 6-MP and weekly oral MTX to standard lymphoma-type maintenance with high dose intermittent Cyclophosphamide and Vincristine-steroid reinforcements in Stage I, II and III disease.

3. To test the efficacy of high dose Methotrexate ( $500 \text{ mg/M}^2$ ) in a maintenance program for patients with Stage IV disease.

Technical Approach: Stages I, II and III Induction Treatment will consist of:

vincristine  $2 \text{ mg/M}^2$  IV x 4 doses given on days 1, 8, 15 and 22

plus

dexamethasone  $6 \text{ mg/M}^2$  p.o. daily x 4 weeks and then taper

plus

methotrexate  $12 \text{ mg/M}^2$  IT given on days 1, 8, 15 and 22

Radiation therapy will begin on day 15.

Maintenance:

Regimen I: cyclophosphamide  $500 \text{ mg/M}^2$  IV push x 1 beginning on day 36 of study and every 4 weeks thereafter

plus

vincristine  $2 \text{ mg/M}^2$  IV push x 1 beginning on day 36 and every 4 weeks thereafter

plus

dexamethasone  $6 \text{ mg/M}^2$  p.o. daily x  
7 days every 4 weeks beginning on  
day 64 of study  
plus  
methotrexate  $15 \text{ mg/M}^2$  p.o. once weekly  
plus  
 $6\text{-MP } 75 \text{ mg/M}^2$  p.o. daily

Regimen II: cyclophosphamide  $1,000 \text{ mg/M}^2$  IV push  
x 1 beginning on day 36 and every  
4 weeks thereafter  
plus  
vincristine  $2 \text{ mg/M}^2$  IV push x 1  
beginning on day 36 and every 4 weeks  
thereafter  
plus  
dexamethasone  $6 \text{ mg/M}^2$  p.o. daily x  
7 days every 4 weeks beginning on  
day 64

#### Treatment of Stage IV Disease - Induction

All Stage IV patients will receive the same  
therapy, consisting of:

vincristine  $2 \text{ mg/M}^2/\text{week}$  IV x 4  
doses given on days 1, 8, 15 and 22  
plus  
dexamethasone  $6 \text{ mg/M}^2$  p.o. daily x  
4 weeks and then taper  
plus  
methotrexate  $12 \text{ mg/M}^2$  IT given on  
days 1, 8, 15 and 22

Radiation therapy will begin on day 15.

#### Intensification

Regimen III: vincristine  $2 \text{ mg/M}^2/\text{week}$  IV x 3 doses  
given on days 36, 57 and 78 of study  
plus  
dexamethasone  $6 \text{ mg/M}^2$  p.o. daily x 1  
week beginning on days 57 and 78  
plus  
methotrexate  $12 \text{ mg/M}^2$  IT given on  
days 36, 57 and 78. IT MTX should be  
given between 1/2 and 2 hours after  
the start of the high dose MTX  
( $500 \text{ mg/M}^2$ )  
plus

methotrexate 500 mg/M<sup>2</sup> 1/3 IV push  
and 2/3 IV drip over 24 hours  
given on days 36, 57 and 78

plus

leucovorin twenty-four hours after  
completion of each course of MTX  
(500 mg/M<sup>2</sup>), leucovorin will be  
given at 12 mg/M<sup>2</sup> IV or IM once  
only as "rescue"

Maintenance therapy will begin on day 85 after the  
completion of the Intensification

Regimen IV: cyclophosphamide 500 mg/M<sup>2</sup> IV push  
x 1 beginning on day 36 of study and  
every 4 weeks thereafter

plus

vincristine 2 mg/M<sup>2</sup> IV push x 1  
beginning on day 36 and every 4 weeks  
thereafter

plus

dexamethasone 6 mg/M<sup>2</sup> p.o. daily x 7  
days every 4 weeks beginning on day  
64

plus

methotrexate 15 mg/M<sup>2</sup> p.o. once weekly

plus

6-MP 75 mg/M<sup>2</sup> p.o. daily

plus

IT Methotrexate 12 mg/M<sup>2</sup> IT given on  
days 36, 43 and 50

The radiation dose is 3500 rads in 3-1/2 to 4 weeks,  
given in 180 to 200 rad fractions.

Progress & Results: WRAMC entered one patient.

No CALGB data are available as of March 1976.

Conclusions: None

Funding Requirements: See introductory remarks to the Annual Research  
Report

Publications: None.

Work Unit No.: 1542

Title of Project: CALGB Protocol #7583. Adjuvant chemotherapy in osteogenic sarcoma, Adriamycin vs. Sequential Adriamycin, High Dose Methotrexate - Citrovorum Factor vs. Sequential Adriamycin - Cyclophosphamide

Investigators:

Principal: Johannes Blom, M.D.

Associate: Frederick B. Ruymann, M.D., LTC, MC

Objectives: 1. To determine the relative duration of disease free interval and survival for patients treated with six courses of Adriamycin alone, or sequential Adriamycin and high dose Methotrexate, followed with citrovorum factor rescue, or sequential Adriamycin and high dose Cyclophosphamide after radical operation of either primary lesion, or complete resection of pulmonary metastasis or osteogenic sarcoma.  
2. To determine the patient's tolerance to these different therapeutic regimens.

Technical Approach: Regimen I: adriamycin 30 mg/M<sup>2</sup> daily for 3 days IV, to be repeated every 4 weeks for 6 courses. The treatment will begin no sooner than 4 days and not later than 4 weeks following operation.

Regimen II: Day 1 to 3, adriamycin 30 mg/M<sup>2</sup> IV daily for 3 days  
Day 28 to 30, adriamycin 30 mg/M<sup>2</sup> IV daily for 3 days  
Day 56, high dose methotrexate 200 mg/kg body weight IV infusion for 6 hrs. Two hours after completion of the high dose MTX infusion, administer citrovorum factor 12 mg intramuscularly every 6 hrs for 12 doses.  
Day 77, high dose methotrexate 200 mg/kg IV infusion for 6 hrs. Two hours after completion of infusion, administer citrovorum factor 12 mg intramuscularly every 6 hrs for 12 doses.  
Day 105, repeat the above adriamycin, high dose MTX plus citrovorum factor sequence at the same dose and interval for a total of 6 courses for each agent

Regimen III: Day 1 to 3, adriamycin 30 mg/M<sup>2</sup> IV  
daily for 3 days  
Day 28 to 30, adriamycin 30 mg/M<sup>2</sup> IV  
daily for 3 days  
Day 56, cyclophosphamide 25 mg/kg  
IV every other day for 5 doses over  
a 10 day period  
Day 98, repeat the above adriamycin,  
cyclophosphamide sequence for a  
total of 6 courses for adriamycin  
and 3 courses for cyclophosphamide.

Progress & Results: WRAMC has not entered any patients.

In March 1976, no data were available from CALGB.

Conclusions: Too early.

Funding Requirements: See introductory remarks to the Annual Research Report.

Publications: None.

Work Unit No.: 1543

Title of Project: CALGB Protocol #7651. A Phase III Study.  
Combination chemotherapy of Stage III and IV  
lymphocytic lymphoma (lymphosarcoma) in adults  
with or without radiotherapy consolidation.  
Induction: Vincristine, Streptonigrin, Prednisone  
Maintenance: Cyclophosphamide

Investigators:

Principal: Johannes Blom, M.D.

- Objectives: 1. To confirm the improvement of remission induction in advanced lymphocytic lymphoma by adding streptonigrin to vincristine and prednisone in this phase.
2. To explore the therapeutic potential of radiation therapy in advanced lymphocytic lymphoma following an initial remission induction with combination chemotherapy by comparing identical chemotherapy maintenance arms, one of which adds radiotherapy to initially involved areas.

Technical Approach: Induction vincristine 1 mg/M<sup>2</sup> IV on days 1, 8, 15, 22, 29 and 36  
plus  
streptonigrin 1 mg/M<sup>2</sup> p.o. spaced over 1 hr on days 1, 8, 15, 22, 29 and 36  
plus  
prednisone 40 mg/M<sup>2</sup> p.o. daily in one dose for 42 days, then tapered by halving the dose every 2 days until the patient is receiving 5 mg/M<sup>2</sup>/day, after 3 days of which it should be stopped

Consolidation and Maintenance Regimens for Patients Who Have Obtained at Least a Partial Remission

Regimen I: Maintenance should begin immediately with:  
cyclophosphamide 1 gm/M<sup>2</sup> IV  
plus  
vincristine 1 mg/M<sup>2</sup> (max. 2 mg) IV  
plus  
prednisone 40 mg/M<sup>2</sup> p.o. daily (in one dose) x 7 days

Regimen II: Patients will receive an interim consolidation phase with chemotherapy and radiotherapy to the areas initially involved at the time of entry to the study, to be followed by maintenance chemotherapy. The map of disease distribution prepared on entry will be used to define the sites of radiotherapy.

Radiation therapy will be given in a dose of 3500 to 4000 rads in 4 weeks to the sites of involvement. The daily dose will vary from 180 to 200 rads.

Progress & Results: WRAMC entered one patient.

At the meeting in March 1976, no CALGB data were available yet.

Conclusions: Too early.

Funding Requirements: See introductory remarks to the Annual Research Report.

Publications: None.

Work Unit No.: 1544

Title of Project: CALGB Protocol #7652. A Phase III Study. Combination chemotherapy of Stage III and IV histiocytic lymphoma (reticulum cell sarcoma) in adults with or without radiotherapy or Adriamycin consolidation.  
Induction: Vincristine, Streptonigrin, Prednisone  
Consolidation: Adriamycin  
Maintenance: Cyclophosphamide

Investigators:

Principal: Johannes Blom, M.D.

- Objectives:
1. To confirm the improvement of remission induction in advanced histiocytic lymphoma by adding streptonigrin to vincristine and prednisone in this phase.
  2. To explore the therapeutic potential of radiation therapy in advanced histiocytic lymphoma following initial remission induction with combination chemotherapy.
  3. To evaluate the benefits of a consolidation phase with Adriamycin.

Technical Approach: The induction program for all patients will consist of:

vincristine 1 mg/ $M^2$  IV on days 1, 8,  
15, 22, 29 and 36  
plus  
streptonigrin 1 mg/ $M^2$  p.o. spaced  
over 1 hour on days 1, 8, 15, 22,  
29 and 36  
plus  
prednisone 40 mg/ $M^2$  p.o. daily in one  
dose for 42 days, then tapered by  
halving the dose every 2 days until  
the patient is receiving 5 mg/ $M^2$   
per day, after 3 days of which it  
should be stopped.

Consolidation and Maintenance will be begun on all patients who have obtained at least a partial remission after 6 weeks of induction.

Regimen I: Patients are begun on maintenance chemotherapy immediately, consisting of cyclophosphamide 1 gm/M<sup>2</sup> IV plus vincristine 1 mg/M<sup>2</sup> plus prednisone 40 mg/M<sup>2</sup> p.o. daily for 7 days.

The first 4 courses are to be given at 3 week intervals, after the 4th course continued every 4 weeks.

Regimen II: Patients will receive consolidation phase with 3 courses of adriamycin, vincristine and prednisone after completion of the 6 week induction phase. Consolidation phase consists of:  
adriamycin 60 mg/M<sup>2</sup> IV q 3 weeks x 3  
plus  
vincristine 1 mg/M<sup>2</sup> IV q 3 weeks x 3  
plus  
prednisone 40 mg/M<sup>2</sup>/day p.o. x 7 days  
q 3 weeks.

Maintenance phase is to be started 3 weeks after the last consolidation course, and will consist of: cyclophosphamide, vincristine and prednisone every 4 weeks, as outlined under Regimen I.

Regimen III: The patient is to receive an interim consolidation phase with chemotherapy and radiotherapy to the areas initially involved at the time of entry into the study, to be followed by maintenance chemotherapy.

The radiotherapy shall be delivered to all areas with known initial involvement which were greater than 2 cm in diameter at time of entry into study. If the total aggregate field area is under 300 sq cm the dose will be 4000 rads in 4-5 weeks. If the fields required measure greater than 300 sq cm, the dose will be 3000 rads in 4-5 weeks. This dose will be delivered in 180 to 200 rad fractions daily.

Progress & Results: WRAMC has entered one patient.

At the recent meeting in March 1976, no CALGB data were available yet.

Conclusions: Too early

Funding Requirements: See introductory remarks to the Annual Research Report.

Publications: None.

BUDGET REQUEST WALTER REED ARMY MEDICAL CENTER

FOR FISCAL YEAR 1977

PROPOSED AND ONGOING STUDIES

This budget request is largely for 50% of the salary for the Project Officer, Dr. Johannes Blom, Chief of the Oncology Section, Hematology-Oncology Service, 100% of the salaries of a hematology technician and of a secretary-typist-data manager. Travel funds are requested to permit our investigators to attend ALGB meetings. Funds are requested to reimburse travel costs of patients who were placed on protocol studies at Walter Reed Army Medical Center who subsequently left the area, and who have to travel significant distances to return to WRAMC for followup visits. During 1975, 21 patients made 71 visits to our clinic for followup, at a total cost of approximately \$14,000. Usually four physicians of the Hematology-Oncology Service, two of the Pediatric Hematology-Oncology Service, and one Radiation Therapist have visited each ALGB meeting. The recent meeting on 18-20 March 1976 was attended by the Assistant Chief of Surgery. Total travel costs for these physicians is projected at \$8,000.

More than 50% of the time of the Project Director is spent in supervising and coordinating activities of the staff, fellows and residents on the Hematology-Oncology Service, to allow as great a number of patients as possible to be placed on ALGB and WRAMC protocols, reviewing records of all patients placed on protocol studies and supervising all other correspondence and administrative activities related to protocol studies. All other personnel of the Hematology-Oncology Service contributing to these protocol studies are military: Chief of Hematology-Oncology Service, three staff physicians, four fellows, two residents; Chief of the Pediatric Hematology-Oncology Service, one staff physician, two fellows, and two residents.

The hematology technician is one of three technicians assigned to the Hematology-Oncology Clinic Laboratory to obtain stat CBCs and platelet counts on all patients who come to the Hematology-Oncology Clinic, to aid in processing all bone marrow aspirates and biopsies, to obtain blood for chemistries and other studies which are processed in the main laboratory, and to perform simple coagulation studies in the Hematology-Oncology Clinic. One of these three technicians' main job is the operation of the cell separator to obtain white cells for patients with severe leukopenia and infections.

The secretary-typist-data manager is involved full time in data gathering and all correspondence concerning Acute Leukemia Group B.

All eligible patients are entered on Acute Leukemia Group B protocols. The total number of patients entered from 1 January until 31st of December 1975 was 55. Attached is a list of patients entered on ALGB protocols during this period of time. It is anticipated that more patients will be placed on ALGB protocols in the coming 2-3 years because protocols for nodular and diffuse lymphomas were recently activated.

Ninety-six patients were entered on WRAMC protocols. Nine patients were entered on protocols in cooperation with Dr. Douglas C. Tormey, Head, Medical Breast Service of the NCI. A list of patients entered on all WRAMC protocols from 1 January until 31 December 1975 is attached.

The following protocols were designed and activated in 1975 and during January - February 1976.

Work Unit No.: 1601

Title of Project: WRAMC Protocol #7207 - Add. #0: Use of pentamidine isethionate in pneumonia caused by or suspected to be caused by pneumocystis carini.

Investigators:

Principal: Johannes Blom, M.D.

Objectives: Treatment of pneumonia caused by or suspected to be caused by pneumocystis carinii with pentamidine isethionate.

Technical Approach: Pentamidine isethionate 4 mg/kg IV for 12-14 days.

Progress & Results: Six patients have been entered, five of whom had good clearing of the infiltrative process. The sixth patient had pulmonary hemorrhage at autopsy and no evidence of pneumocystis.

Conclusion: As has been demonstrated by many investigators, pentamidine is an effective drug in the treatment of pneumocystis carinii pneumonia.

Funding Requirements: See introductory remarks to Annual Research Report.

Publications: None.

Work Unit No.: 1602

Title of Project: WRAMC Protocol #7301 - Add. #0: The use of cholestyramine in metastatic carcinoma of the prostate and ovary and other malignancies.

Investigators:

Principal: Johannes Blom, M.D.

Objectives: To observe response in metastatic carcinoma of the prostate and ovary and other malignancies.

Technical Approach: Cholestyramine (questran) 4 gm (one packet) placed in a preferred beverage three times daily. Because of interference with the absorption of lipid soluble vitamins, 2 ml of polyvisol will be administered daily.

Progress & Results: Four patients have been entered on study. Three had no response or progressive disease. One has had minimally progressive disease for about 18 months. One patient was recently entered who had subjective improvement.

Conclusions: Too early.

Funding Requirements: See introductory remarks to Annual Research Report.

Publications: None.

Work Unit No.: 1603

Title of Project: WRAMC Protocol #7206 - Add. #0: The use of methyl-CCNU (1-(2-chloroethyl)-3(4-methylcyclohexyl)-1-nitrosourea) (NSC 95441) in the treatment of brain tumors.

Methyl-CCNU is a nitrosourea derivative related to CCNU with pronounced antitumor activity in a variety of animal and human tumors. Methyl-CCNU like BCNU and CCNU, is relatively unionized at physiologic pH and is highly lipid soluble. Because of these characteristics, these drugs diffuse easily across the blood brain barrier, therefore, nitrosoureas are able to penetrate the actively growing edge of brain tumors, which is most susceptible to cell kill. Encouraging results in the treatment of human malignant brain tumors have been obtained with BCNU in combination with radiation therapy. Methyl-CCNU, which is even more lipid soluble than BCNU and CCNU, may, therefore, be even more active. The treatment schedule is Methyl-CCNU, 150 mg/m<sup>2</sup> p.o in a single dose every 6 weeks. Thirty-six patients have been entered: 16 patients are still on study; 20 patients have expired or have exited from the protocol because of progressive disease. Survival time of these patients will be compared with patients, treated previously without Methyl-CCNU.

Work Unit No.: 1604

Title of Project: WRAMC Protocol #7205 - Add. #0: Phase II  
Protocol Combination Chemotherapy with  
Dimethyl-Triazeno Imidazole Carboxamide (DIC)  
and Adriamycin in Soft Tissue and Bone  
Sarcomas.

Sarcomas in general are rather chemotherapy resistant. Both Adriamycin and DIC have shown some activity. A trial with combination of these two drugs is, therefore, rational. The treatment regimen consists of: A - good risk patients Adriamycin 60 mg/m<sup>2</sup> once every three weeks; DIC, 250 mg/m<sup>2</sup> for 5 days I.V., to be repeated every three weeks; B - poor risk patients Adriamycin 45mg/m<sup>2</sup> on day 1 and DIC 200 mg/m<sup>2</sup> for 5 days I.V. to be repeated every 3 weeks. Twenty-six patients have been entered. Twelve patients are still on study: one patient had a complete remission; one a partial remission; three had no change; six had progressive disease; two patients were not evaluable; and one was lost to followup.

Work Unit No.: 1605

Title of Project: WRAMC Protocol #7204 (EST 2372) - Add. #0:  
Comparison of the Treatment of Metastatic  
Reticular Tumors with Actinomycin-D, or  
Actinomycin-D, Bleomycin and Vincristine.

This is a protocol of the Eastern Cooperative Oncology Group, which was activated in October 1972, and closed to patient entry 25 November 1975. Dr. Blom is the Chairman of this study. Approximately 110 patients have been entered; 7 at WRAMC. A report of the status of this study was presented to the meetings of the American Society of Clinical Oncology in May 1975 in San Diego, and a followup report has been accepted for the same meeting in Toronto this coming May.

Work Unit No.: 1608

Title of Project: WRAMC Protocol #7302 - Add. #0: Treatment of Metastatic Malignant Melanoma with a Combination of Imidazole Carboxamide Dimethyl-Triazeno (ICDT), Methyl-CCNU and Vincristine

The use of single antineoplastic drugs in the treatment of metastatic malignant melanoma has produced response rates of about 15-25%. One of the most promising agents, ICDT, in one study allowed a median survival in responders of 6.5 months, as opposed to 2.8 months in nonresponders. A combination of ICDT and BCNU improved the median survival of responders to 7.1 months. In one study, 10 of 16 patients with melanoma treated with ICDT, BCNU and Vincristine showed significant response. In this protocol, Methyl-CCNU is used because it also has considerable antitumor activity and it can be given by mouth, as opposed to BCNU, which must be given intravenously. The treatment schedule is ICDT, 600 mg/m<sup>2</sup> I.V. q 6 weeks; Methyl-CCNU 100 mg/m<sup>2</sup> p.o. q 6 weeks; and Vincristine, 2 mg I.V. every other week. Twenty patients have been entered on study: 2 are unevaluable; 3 had a complete remission; 1 had a partial remission; 1 had improvement; 5 had no change; 4 had progressive disease. Four patients are still on study.

Work Unit No.: 1609

Title of Project: WRGH #707 - Add. #0: Comparative Study of Synchronizing - Chemotherapy in Adult Solid Tumors Utilizing Methotrexate (With Citrovorum Factor Reversal). Followed by Cytosine Arabinoside and 1,3-Bis-(2-Chlorethyl)-Nitrosourea

No. 1

Name: Schupe, William

Diagnosis: All

Date: 3 Jul 75

Work Unit No.: 1610

Title of Project: WRAMC Protocol #7307 (NCI B-134) - Add. #0:  
Phase I-II Evaluation of Dibromodulcitol  
in Previously Treated Patients with  
Metastatic Carcinoma of the Breast

Three patients have been entered: two patients had progressive disease; one patient is still on study.

Work Unit No.: 1611

Title of Project: WRAMC Protocol #7403 - Add. #0: Treatment of Advanced Lung Cancer with a Combination of 1,2-di(3,5-dioxypiperazine-1-yl-propane) (ICRF 159) and Adriamycin with Cyclophosphamide Maintenance.

The treatment of lung cancer with single chemotherapeutic agents has in general at best only added a slight prolongation in survival. Combination chemotherapy has given much improved response rates in leukemia, lymphoma and breast carcinoma. Further trials with combination chemotherapy for solid tumors are needed to improve response rates. Adriamycin, a relatively new antineoplastic drug, has been shown to have a partial response rate of 19% in 229 patients treated for bronchogenic carcinoma. It has been shown to be effective in epidermoid, large cell undifferentiated, adenocarcinoma and small cell (oat cell) carcinoma of the lung. ICRF 159 is another new antineoplastic drug, which in animal studies has been shown to prevent the implantation of metastasis, presumably by inhibiting neovascularization. A combination of ICRF 159 and Adriamycin may be advantageous as a combination in the therapy of bronchogenic carcinoma. Cyclophosphamide is a frequently used antineoplastic agent of the alkylating type, and has been shown to be of some benefit in the treatment of carcinoma of the lung. The treatment schedule consists of Adriamycin, 45 mg/m<sup>2</sup> I.V. every 4 weeks plus ICRF 159, 250 mg/m<sup>2</sup> p.o. every week. The maintenance phase, which is begun when a total limiting dose of Adriamycin of 550 mg/m<sup>2</sup> is reached, consists of ICRF 159, 300 mg/m<sup>2</sup> p.o. q 8 hours for 3 days every 28 days plus Cytoxan, 1.2 mg/m<sup>2</sup> I.V. on day 1 every 28 days. Patients who have had and have become resistant to Cytoxan will receive ICRF 159, 300 mg/m<sup>2</sup> p.o. q 8 hours for 3 days every 28 days.

Eighteen patients have been placed on study; one is still on study. Three were not evaluable; one had no change; and 13 had progressive disease. Although these results are very disappointing, the study will be continued, because most of these patients had far advanced disease when they were entered on the study.

Work Unit No.: 1612

Title of Project: WRAMC Protocol #7401 - Add. #0. Treatment of Advanced Lung Cancer with a Combination of Emetine (NSC 33669) and Cyclophosphamide or 1-(2-chloreethyl)-3-cyclohexyl-1-nitrorourea (CCNU) NSC 79037)

The treatment of advanced lung cancer with single agent chemotherapy has allowed at best only a slight prolongation in survival. The improved response rates and survival seen with combination chemotherapy regimens in acute leukemias, lymphomas and breast carcinomas emphasize the need for further trials in solid tumors previously considered resistant to cure. Phase I studies with Emetine hydrochloride (NSC 33669) as an antitumor agent revealed that the drug is not myelosuppressive, and, therefore, may be useful in the patient with poor marrow reserve. Alternatively, Emetine could be combined with agents of different toxicities, in hope of showing additive effects without additive toxicity. The combination of cyclophosphamide and Emetine allowed excellent responses in several types of lung cancer, as reported by Street. CCNU is another promising single agent in the treatment of lung cancer, and data from studies with solid tumors in animals point to the effectiveness of CCNU in combination with Emetine. Treatment schedules are Cyclophosphamide, 1.2 gm/m<sup>2</sup> I.V. every 3 weeks plus Emetine, 2 mg/kg I.V. every week for 6 weeks; or CCNU, 100 mg/m<sup>2</sup> p.o. every 6 weeks plus Emetine, 2 mg/kg I.V. every week for 6 weeks. The protocol was discontinued in January 1976. Twelve patients were entered on study; three were not evaluable; one was disqualified; six had progressive disease; and two had no change. Because of the disappointing results, the study was discontinued. All these patients hav previous chemotherapy, and were in the final stage of their disease however.

Work Unit No.: 1613

Title of Project: WRAMC Protocol #7402 - Add. #0: Adjuvant Therapy of Stage II Testicular Carcinoma with Chemotherapy, Radiation Therapy or Chemotherapy Plus Radiation Therapy after Retroperitoneal Lymph Node Dissection.

This protocol of the testicular tumor study was activated in January 1974, however, it was deactivated on the 26th of January 1976. Four patients were entered at this hospital; two received chemotherapy and two received radiation therapy. The patients with chemotherapy had recurrent disease; the patients with radiation therapy are still in remission.

During 1975, eight patients with testicular tumors were treated with Cis-Platinum in several combinations. Five of these patients had received prior chemotherapy; three had not received any previous therapy; two of these three were treated with the VAB-II program, consisting of Actinomycin-D and Velban on day 1, and Bleomycin, daily infusions, for 7 days, followed by Cis-Platinum, 1 mg/kg on day 8. One patient had a good regression of retroperitoneal nodes, but because of residual disease, he was begun on radiation therapy to the periaortic nodes and laparotomy with possible lymph node dissection was planned upon completion of the radiation therapy. After this, the chemotherapy will be continued.

The second patient had a greater than 50% regression of pulmonary lesions, however, he left WRAMC and did not return to the local VA Hospital for further care until three months later, at which time he had recurrent disease. The third previously untreated patient had widespread disease and was treated with the VAB-III treatment program, as is being used at Memorial Hospital in New York, and consisting of Cytoxan, Velban, Actinomycin-D given on day 1, Bleomycin daily infusions for 7 days, followed by 3.1 mg/kg of Cis-Platinum, plus Mannitol infusion. Maintenance courses are with Velban, Actinomycin-D plus Chlorambucil, and Cis-Platinum, Velban plus Chlorambucil. After the first course of treatment, patient has a complete regression of all measurable disease. He is presently in excellent condition and continues on the protocol. New Patients will be entered on this same VAB-III program.

Work Unit No.: 1617

Title of Project: WRAMC Protocol #7105 - Add. #0: Phase II  
Study of the Combined Use of Bleomycin in  
the Treatment of Adult Malignancies

No.: 1

Name: DeVary, William

Diagnosis: Hepatoma

Date: 15 Sept 75

Work Unit No.: 1619

Title of Project: Non-Protocol Use of All Drugs

Work Unit No.: 1620

Title of Project: WRAMC Protocol #7305 - Add. #0: Study to  
Evaluate the Effect of Estrogen Therapy  
with and without Additive Chemotherapy in  
the Management of Recurrent Mammary  
Carcinoma in Post-Menopausal Women  
(NCI B-124)

Four patients were entered, three had progressive disease, and  
one has no change.

Work Unit No.: 1621

Title of Project: WRGH Protocol #7208 - Add. #0: Phase II  
Study, 5-Azacytidine in Acute Leukemia

5-Azacytidine is a pyrimidine nucleotide analogue, produced  
microbiologically from the *Streptoverticillium* species. This  
agent has shown substantial activity against mouse L1210  
leukemia and AKR transplant mouse leukemia. Preliminary  
responses in humans reported from Europe were encouraging. In  
the United States, 13 patients with ALL had no response, and  
22% of 9 patients with AML had a complete response.

An additional 3 patients achieved a partial remission. The treatment schedule is  $250 \text{ mg/m}^2$  intravenously daily for 5 days. Seven patients with AML and one patient with a blastic crisis of CML were entered. One patient had a partial response; none of the others responded.

Work Unit No.: 1625

Title of Project: WRAMC Protocol #7304 - Add. #0: Study to Evaluate the Effect of Oophorectomy with and without Adjuvant Chemotherapy in the Management of Recurrent Mammary Carcinoma in Pre-Menopausal Women

Three patients have been entered; none had a response.

Work Unit No.: 1626

Title of Project: WRAMC Protocol #7405 - Add. #0: Treatment of Advanced Renal Cell Carcinoma with a Combination of 1-(Chlorethyl)-3-Cyclohexyl-1-Nitrosourea (CCNU) (NSC #79037) & Bleomycin (NSC #125066)

Treatment of metastatic carcinoma of the kidney with chemotherapy has been generally disappointing. Tally and associates have recently documented the ineffectiveness of a wide variety of chemotherapeutic agents. Progestational and adrogenic substances have had minimal results in humans. Merrin and associates recently reported a 10% objective response and 50% symptomatic improvement with arrest of disease progression for a period of up to 10 months with a combination of CCNU and Vinblastine. The treatment in this protocol consists of CCNU given  $130 \text{ mg/m}^2$  every 6 weeks, and Bleomycin, 15 mg once a week for induction, with Bleomycin to be decreased to every 3 weeks for maintenance, total dose not to exceed  $210 \text{ mg/m}^2$ . All patients with histologically proven inoperable primary renal carcinoma and/or metastatic carcinoma will be included in the study after they have recovered from all beneficial side-effects of previous treatment.

Also, included will be patients who, at time of surgery, are found to have disease infiltrating through the renal capsule, disease outside renal capsule, and/or disease in the blood vessels. Fourteen patients have been entered on the protocol: 7 had clinical evidence of metastatic disease, and all 7 patients had progressive disease on treatment; 2 patients with metastatic disease are still on study; 5 patients with adjuvant treatment were entered, 1 of these expired on day 175, the other 4 are still free of disease, varying from 119 to 516 days.

Work Unit No.: 1627

Title of Project: WRAMC Protocol #7404 - Add. #0: Immunological Evaluation and Immunotherapy of Patients with Carcinoma of the Lung

This is a study in cooperation with the Cellular and Tumor Immunology Section, Laboratory of Cell Biology, National Cancer Institute. The protocol was activated in August 1974. Bethesda Naval Hospital and Portsmouth Naval Hospital also contribute patients to this study. Because of the positive results in the course of solid neoplasia in experimental animals and recently reported successes with immunotherapy of ALL, AML and melanoma in humans, it seemed appropriate to begin clinical trials of immunotherapy in human solid tumors at WRAMC.

The lack of success in a previous trial in human lung carcinoma may be attributed to a variety of causes, the most important of which would seem to be far advanced state of disease in the patients with a presence of a large tumor load. Animal experimental studies suggest that immunotherapy may be more effective when the tumor load is small.. Carcinoma of the lung in the human would seem to be a proper system in which to study the effect of immunotherapy: (1) the yearly death rate from this neoplasm is increasing; (2) there has been no essential improvement in prognosis; (3) since survival is short, a rapid assessment of results will be possible; (4) patients with intact cellular immunity may respond better to chemotherapy; (5) patients with carcinoma of the lung may have intact cellular immunity, as measured by DNCB skin testing; (6) there is some evidence that cancer of the lung possesses a tumor specific antigen.

Frequently the disease is resectable by surgery or treatable with radiotherapy with control of the primary neoplasm, but the patient dies of metastatic disease. Because metastatic disease appears quickly in these patients, it may be that it was present but clinically undetected at the time of primary therapy. These patients after surgery should have a small tumor load, may be amendable to immunotherapy, as has been suggested in animal systems and human acute leukemia. A similar protocol might be applied to those patients with metastatic disease at the time of diagnosis, but with much less hope of success. Of the various possibilities for immunotherapy, two are to be studied in this protocol: (1) BCG and (2) Allogeneic tumor cells. BCG has been shown to be an immunostimulant in animal tumor systems, decreasing the number of "takes" of transplantable tumors, as well as decreasing the incidence of spontaneous viral leukemia. Successful clinical trials have been reported for ALL, AML, and for melanoma.

Because BCG by dermal scar application seems to be an effective immunostimulant and is accompanied by less than desirable side effects (i.e., ulceration and occasional severe reactions) than intralesional BCG, the former is the method of choice. Allogeneic cells have been shown to have an additive effect to BCG in animal systems, and have been used in the immunotherapy of acute leukemia. Allogeneic cells provide a source of tumor associated antigen and the BCG acts as an adjuvant to help the lymphoid system respond to such immunization.

All patients with primary carcinoma of the lung are eligible for this study. The patients can be put into three broad groups, based on the extent of disease. A patients - surgically resectable disease (no clinically detectable tumor after surgery). B patients - (1) Surgically treatable for the bulk of tumor, but not completely locally resectable (palliative resection). (2) Residual disease treatable by local radiotherapy (after 1) or primary disease treatable by radiotherapy (patients in whom surgery is contraindicated). C Patients - Patients with metastatic disease.

A patients will have surgical removal of tumor, primary and regional exploration of the area for local metastasis in the standard operative manner. These patients will not receive any chemotherapy, but will be randomized between immunotherapy and no immunotherapy.

All B patients who are found to have unresectable disease, either at surgery or prior to surgery, are candidates for local radiation therapy which can be in a curative intent with about 5000 rads in 5 weeks. In addition, the B patients will receive chemotherapy, as will be outlined below, and will be randomized between no immunotherapy, the control group, or non-specific active immunotherapy BCG only, and specific active immunotherapy, BCG plus allogeneic cells. C patients may receive palliative radiation therapy to the site of the tumor and to local tumor masses during chemotherapy for metastatic disease if clinical systems warrant. C patients will be treated with chemotherapy, consisting of Cytosine Arabinoside, Methotrexate, and Vincristine given on days 1 and 8 of a 21 day cycle. The chemotherapy will be continued for a 2 year period. In addition, these patients will be randomized into three groups: (1) Control group—no immunotherapy. (2) Nonspecific active immunotherapy with BCG only. (3) Specific active immunotherapy with BCG and allogeneic cells. Thirty-two patients were entered at WRAMC. The study was discontinued in December 1975. Reports are in the final stages for publication.

At a meeting of The Surgeon General's Army Investigational Drug Review Board (AIDRB) on 4 March 1975, the Board upheld approval of this protocol until the investigator had provided written verification that an IND had been submitted, as requested, or provide evidence from the Bureau of Biologics that the FDA does not require an IND. Because of various circumstances, investigators at the National Cancer Institute delayed the requisition for such an IND. In the interim, patients were entered into the protocol, but the regimen containing the irradiated tumor cells was dropped from the regimen at WRAMC. The protocol was closed to entry of patients, with the exception of Stage I patients, in December 1975. Stage I patients are randomized between BCG and control.

Work Unit No.: 1628

Title of Project: WRAMC #7406 - Add. #0: Chemoimmunotherapy  
of Carcinoma of the Large Bowel

Activated September 1974. Cancer of the large bowel is the second most common solid malignant neoplasm in man, accounting for about 7% of all cancers. Improvement in our detection and surgical technique has increased 5-year survival, but, over the last decade, this improvement in survival has reached a plateau, while a distressingly large number of patients still succumb to their disease. The use of adjuvant chemotherapy, or of preoperative radiotherapy, has not, at this time, been shown definitively to effect 5-year survival when compared to surgery alone; and although there is reason to believe these methods may be proven to be beneficial, significant number of patients will still die of their disease. Thus, there remains the need to evaluate other modes of therapy to complement the partial success of surgery, chemotherapy and radiotherapy. Patients with carcinoma of the colon or rectum will be eligible for entry into the study, except patients with disease limited to the mucosa or extending to, but not beyond, the muscularis, with negative lymph nodes. Patients eligible for this protocol can be put into four broad groups based on the extent of disease: A Patients: Disease confined to the bowel wall - totally resectable. B Patients: (1) Patients with disease involving regional lymphatics, but totally resectable, (2) Patients with more extensive local metastasis, but totally resectable (e.g., involving a viscous proximal to the tumor which can be removed leaving no residual tumor). C Patients: (1) Patients with locally metastatic disease beyond lymphatics, the bulk of which can be removed, but with some tumor remaining. (2) Patients who cannot tolerate surgery. (3) Patients whose tumor is of such size or fixed so that surgery would not be undertaken. D Patients: Patients with distant metastasis.

A and B patients will receive 5-FU by mouth for 5 days, every 28 days. In addition, A and B patients will be randomized between immunotherapy with BCG and no immunotherapy. This will be administered on days 8, 15 and 22 of the chemotherapy cycle for three courses, and then every two weeks on days 8 and 22 thereafter, for at least two years. C (1) patients, about 3 weeks after surgery, will be treated as D patients. C (2) patients, after radiation therapy, will be treated as D patients. C (3) patients, if after radiotherapy this patient is operable and tumor is completely resectable, the patient will begin chemotherapy and a B patient. If the tumor is not completely resectable, they will be treated as D patients. If, after radiotherapy, the patient is felt to be inoperable, he will be treated as a D patient. D patients will be treated with 5-FU, 30 mg/kg I.V. daily as a 24 hour infusion for 5 days, with courses repeated each 21 days for 3 courses.

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CLINICAL INVESTIGATION SERVICE. (U)  
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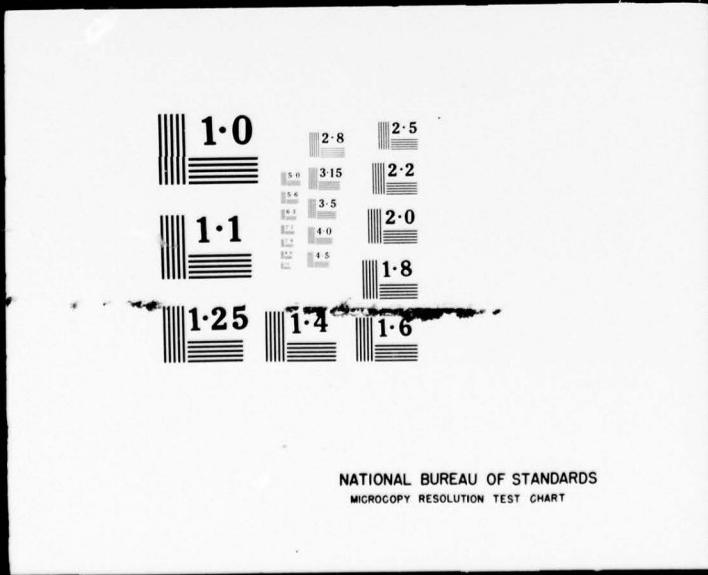
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They are then treated as A and B patients. Fifteen patients have been entered on the protocol: 2 were disqualified; 3 have been metastatic disease and are still on study; 10 had no residual disease and were treated with the adjuvant regimen. Their times on study vary from 63 to 461 days, and all are still free of disease.

Work Unit No.: 1629

Title of Project: WRAMC Protocol #7407 - Add. #0:  
Chemoimmunotherapy of Malignant Melanoma

Activated September 1974. The rationale for early adjuvant chemoimmunotherapy has been outlined in the previous protocols. In this protocol, patients with malignant melanoma, Stage I, will be treated with BCG, and patients with Stage II-IV with chemotherapy and BCG. In March, the protocol was addended, excluding patients with Stage I extremity lesions from entry into the protocol when the lesion does not penetrate into or beyond the reticular layer of skin, because these have a better survival than patients whose lesions do penetrate into or beyond the reticular layer of skin (Clark's level IV or V). Patients with Stage I disease will be treated with BCG by dermal scarification weekly for 3 months, then every other week for 21 months. The chemotherapy for patients with Stage II, III and IV will consist of ICDT, 700 mg/m<sup>2</sup> given as an I.V. push on day 1 or a 21 day cycle; BCG will be administered on days 7, 12 and 17 of a 21 day cycle. This treatment will be continued for at least two years after complete remission, or, if complete remission is not achieved, until there is evidence of progressive disease. Fourteen patients were entered on study: 4 had progressive disease; 10 are still on study, varying from 75 to 411 days.

Work Unit No.: 1630

Title of Project: WRAMC Protocol #7408 - Add. #0: Comparative Trial of Tamoxifen and Fluoxymesterone  
Plus Tamoxifen in Metastatic Breast Cancer

Three patients have been entered, one of whom was non-random, and had only some improvement. One patient had progressive disease, and one patient had a partial remission.

Work Unit No.: 1631

Title of Project: WRAMC Protocol #7409 (NCI B142) - Add. #0:  
Phase I-II Evaluation of a Combination of  
BCNU and Methotrexate in Metastatic  
Breast Cancer

No patients have been entered.

Work Unit No.: 1632

Title of Project: WRAMC Protocol #7410 - Add. #0: Combination  
of MeCCNU plus VP 16-213 in the Treatment  
of Metastatic Adenocarcinoma of the  
Gastrointestinal Tract and the Pancreas

This is a pilot study for the Acute Leukemia Group B. In an ALGB study which is presently active, there were some responses to VP 16-213 in carcinoma of the colon and pancreas. Also, Methyl-CCNU has been found to have activity in GI malignancies. A trial with a combination seems, therefore, rationale. Methyl-CCNU is given  $150 \text{ mg}/\text{m}^2$  once every 6 weeks; VP 16-213  $60 \text{ mg}/\text{m}^2$  I.V. twice weekly. Two patients have been entered on the protocol: the first patient had marked necrosis of a large cutaneous tumor mass, but expired from inanition on day 18, and the second patient had one course of therapy, but had progressive disease. Because of lack of response, the study was discontinued in the Acute Leukemia Group B.

Work Unit No.: 1633

Title of Project: WRAMC Protocol #7411 - Add. #0: (NCI B135)  
Evaluation of a Combined Modality Approach  
to Therapy in Mammary Carcinoma Patients  
with Tc 1,2,3a,4b N+ Lesions: Comparative  
Study of: Radical Mastectomy, Cyclophosphamide,  
Methotrexate and 5-Fluorouracil versus  
Radical Mastectomy, Cyclophosphamide,  
Methotrexate 5-Fluorouracil, C. parvum  
versus Radical Mastectomy

One patient has been entered on this study. The patient  
developed metastatic lesions and was removed from protocol.

Work Unit No.: 1634

Title of Project: WRAMC Protocol #7412 - Add. #0: Metastatic  
Breast Carcinoma Study to Evaluate the  
Effect of Cyclophosphamide, Adriamycin  
and 5-Fluorouracil Versus Adriamycin,  
Dibromodulcitol and Vincristine Sequentially  
Alternating with Cyclophosphamide,  
Methotrexate and 5-Fluorouracil

Eight patients were entered. One patient had improvement, but  
died 8 months later with progressive disease. Seven patients  
are still on study, varying from 40 days to 426 days. The  
patient who has been on study for 426 days obtained a complete  
remission.

During 1975, a WRAMC Fellow completed a research project with  
NCI as an elective project during his second year Fellowship.  
Dr. Heim, who after completion of his Fellowship in July  
became a staff member, continued to donate most of his time  
to the chemo-immunotherapy projects as outlined under Protocol  
#7404 (lung carcinoma), #7406 (colorectal carcinoma), #7407  
(melanoma), and #7601 (lung carcinoma). One Fellow from the  
NCI Washington VA Hospital Oncology Service spent the months  
of January through June on our Hematology-Oncology Service as  
an elective and one Fellow the months of April and May. Both  
of these programs have continued to contribute greatly to our  
service and to the persons involved.

Work Unit No.: 1642

Title of Project: An investigation conducted by the Microbiology  
Department of the University of Maryland

Investigators:

Principal: Lynn Wilson, Graduate Student, University of Maryland

Associate: Johannes Blom, M.D.

Objectives: This study was undertaken to look at the degree of immunologic recognition invoked in peripheral lymphocytes from infectious mononucleosis, Hodgkin's disease, and American Burkitt's lymphoma patients on challenge by EBV antigen.

Technical Approach: The level of immunologic recognition will be measured by inhibition of macrophage migration from capillary tubes (MIF), i.e., the level of MIF produced by the challenge cells. Preliminary data from infectious mononucleosis challenges indicates an average level of inhibition of 25%, which is significantly greater than normal controls.

Progress & Results: Because of logistic problems, only a few specimens have been forwarded to the University of Maryland for examination.

Conclusions: None

Funding Requirements: None

Publications: None

Work Unit No.: 1644

Title of Project: WRAMC Protocol #7501 - Add. #0: Evaluation of Adriamycin and cis-Platinum Combination Chemotherapy in Treatment of Malignant Disease

A Phase II Study. Adriamycin has been found to be highly effective in the treatment of sarcomas, carcinoma of the breast, bronchogenic carcinoma, Hodgkin's disease, non-Hodgkin's lymphomas, acute myelocytic and acute lymphocytic leukemias, thyroid carcinoma, bladder carcinoma and neuroblastoma. Cis-Platinum has been found to have activity in head and neck epidermoid carcinomas, sarcomas, Hodgkin's disease, non-Hodgkin's lymphomas, adenocarcinoma of the breast, and especially testicular germ cell tumors. This protocol is for the evaluation of the antitumor activity of the combination of Adriamycin and Cis-Platinum in previously untreated malignancies that have a low order of response to conventional modes of therapy, such as head and neck carcinoma, squamous and adenocarcinoma of the lung, metastatic transitional cell carcinoma of the bladder, renal cell carcinoma, etc. Evaluation of antitumor activity of this combination in malignancies that have become refractory to conventional modes of therapy, such as ALL, AML, Hodgkin's disease, non-Hodgkin's lymphoma, oat cell carcinoma of the lung, adenocarcinoma of the prostate, soft tissue sarcoma, multiple myeloma, etc. The treatment schedule consists of Adriamycin 60 mg/m<sup>2</sup> intravenously day 1 every 21 days and Cis-Platinum 60 mg/m<sup>2</sup> IV day 1 every 21 days.

To date, eight patients have been placed on study; two patients had progressive disease; the remainder are still on study.

Work Unit No.: 1646

Title of Project: WRAMC Protocol #7503 - Add. #0: Clinical Evaluation of Galactitol 1,2:5,6-Dianhydro-(NSC 132313) in the Treatment of Metastatic Renal Cell Carcinoma. A Phase II Study.

The treatment results of metastatic renal cell carcinoma have been dismal. A number of hormonal and cytotoxic agents have been utilized but with equivocal results. Galactitol is the active metabolite of Dibromodulcitol. The latter agent has had a limited trial in renal cell carcinoma, with an overall response rate of 21% (4 of 19 patients). Galactitol itself was used in five patients with renal cell carcinoma, with a partial response in 40% (2 of 5). The treatment regimen consists of 60 mg/m<sup>2</sup> intravenously every 7 days. No patients have been entered to date.

Work Unit No.: 1647

Title of Project: Inhibition of red cell pyridoxal kinase by the carbonyl reagents, isoniazid and hydralazine

Investigators:

Principal: MAJ John A. Kark, M.D., MC  
MAJ Thomas Gibson, M.D., MC  
Associate: LTC Michael J. Haut, M.D., MC  
MAJ William Babcock, M.D., MC  
Peter Tarassoff, George Washington Univ School Med  
Kenneth Goldstein, M.D., George Washington Univ  
School Med

Objectives: To define the time course of onset of inhibition of this enzyme during conventional therapy with isoniazid or hydralazine, to elucidate the mechanism of the inhibition, and to determine the time course of decline of cellular coenzyme-B6 which results from these medications.

Technical Approach: This has been outlined in detail in our clinical investigation protocol. We will assay red cell pyridoxal kinase activity, using lightly washed red cell suspensions with very low levels of pyridoxal as substrate, to detect the presence of competitive inhibitors. Subjects will be studied before taking the medications (isoniazid or hydralazine) and during the time of treatment. Levels of plasma and red cell pyridoxal phosphate will also be followed to detect changes induced by these drugs.

Progress: Only one subject has been studied during the day preceding and the first 3 days of administration of INH, 300 mg p.o. No significant change in erythrocyte pyridoxal kinase activity was detected at high concentrations of substrate. In future studies a low level of substrate (below the  $K_m$  concentration) will also be used, since this will be more likely to demonstrate the presence of a partially competitive inhibitor. The assistance of a medical student, Mr. Peter Tarrassof, has been obtained for the coming summer.

Conclusions: This work is just beginning, and there are obviously no significant results or conclusions at this time.

Funds Utilized, FY 76: None

Funding Requirements, FY 77:

Personnel: None

Equipment: None

Supplies: None

Travel: \$250 for presentation of data at a meeting

Publications: None

Type of Report: Interim

Work Unit No.: 1649

Title of Project: WRAMC Protocol #7602 - Add. #0:  
Chemotherapy of Prostatic Carcinoma

Carcinoma of the prostate is the fourth leading cause of cancer in men. The treatment of this disease is unsatisfactory: only 5% of patients are candidates for radical prostatectomy. Radiation therapy has been utilized to treat locally advanced but nonresectable disease, but with dissemination its use is essentially limited to palliation. For those patients who fail to respond to hormonal manipulation, or those who relapse after initial response, the outlook is poor. Limited chemotherapeutic trials have shown little or no response to most standard agents. Current studies are underway comparing Cyclophosphamide, 5-Fluorouracil, and Adriamycin in varying doses and combination in the treatment of advanced metastatic prostatic carcinoma. Reported response rates with these agents have varied from 20-40%. Recent studies have shown that a number of human malignancies respond to immunotherapy, and that the depression of cell mediated immunity may play a significant role in the growth and dissemination of malignant tumors. In one study, objective local tumor regression was demonstrated following intraprostatic injections of BCG in patients with metastatic carcinoma of the prostate. Patients with histologically proven prostatic carcinoma with distant metastasis, who have either relapsed or have been unresponsive to hormonal manipulation, will be eligible for inclusion in this study. Patients will randomly be assigned to treatment with chemotherapy or chemotherapy plus immunotherapy. The chemotherapy will consist of Cyclophosphamide, 100 mg/m<sup>2</sup> I.V. given on day 1; 5-Fluorouracil, 600 mg/m<sup>2</sup> I.V. given on days 1 and 8, to be repeated every 28 days. The immunotherapy will consist of BCG, 6 x 10<sup>8</sup> units on days 14 and 2. To date, one patient has been entered on the study.

Work Unit No.: 1650

Title of Project: WRAMC Protocol #7603 - Add. #0: Evaluation of Galactitol 1,2:5,6-Dianhydro in the Treatment of Advanced Neoplastic Disease

A Phase II Study. In several institutions, clinical studies of Galactitol have shown some responses in patients with squamous cell, small cell and adenocarcinoma of the lung, renal cell carcinoma and melanoma, but the number of patients has been too small to draw conclusions. Therefore, we plan to determine the antitumor effect of Galactitol in a broad spectrum of metastatic tumors, mostly patients with histologically proven malignancies that are refractory to treatment with drugs of proven efficacy. The treatment schedule is 60 mg/m<sup>2</sup> given I.V. every 7 days. One patient has been entered to date.

Work Unit No.: 1651

Title of Project: WRAMC Protocol #7604 - Add. #0: Combination Chemotherapy for the Treatment of Advanced Gastric Carcinoma with Either 1-(Tetrahydro-2-Furanyl)-5-Fluorouracil (Ftorafur), Adriamycin and Mitomycin-C vs 5-Fluorouracil, Adriamycin and Mitomycin-C

Despite a decrease in the incidence of gastric carcinoma in the United States in the past 30 years, this disease still remains the sixth leading cause of cancer death in this country. The survival of patients with unresectable disease is poor. Responses to single agent chemotherapy have been disappointing in its effectiveness. 5-Fluorouracil, Mitomycin-C, BCNU and Adriamycin have been found to have activity in this malignancy. Combination studies are presently underway in different institutions. This study was originated by the medical oncology division at Georgetown University. Patients will be randomized between two treatment schedules. Schedule A: Ftorafur, 1000 mg/m<sup>2</sup> per day I.V. for 5 days, to be given weeks 1 and 5 of an 8 week cycle. Mitomycin-C, 10 mg/m<sup>2</sup> I.V. every 8 weeks. Adriamycin, 40 mg/m<sup>2</sup> every 4 weeks. Schedule B: Fluorouracil, 600 mg/m<sup>2</sup> days 1, 8, 29 and 36. Mitomycin-C, 10 mg/m<sup>2</sup> I.V. every 8 weeks. Adriamycin, 40 mg/m<sup>2</sup> every 4 weeks. Two patients have been entered to date.

Work Unit No.: 1652

Title of Project: WRAMC Protocol #7605 - Add. #0: Combination Chemotherapy of Advanced Pancreatic Carcinoma with 5-Fluorouracil, Mitomycin-C and Streptozotocin

Carcinoma of the pancreas has been increasing in prevalence during the last two decades. This tumor now ranks fourth as a cause of cancer deaths in the U.S. Long-term survival in advanced disease is exceedingly rare, and median survival varies between 2-6 months, with only 2% survival at 24 months. Little data is currently available on the activity of individual chemotherapeutic agents in this disease, however, 5-Fluorouracil, Mitomycin-C and Streptozotocin have all been shown to produce objective responses in 15-30% of patients.

Combination chemotherapy is now beginning to be explored in a randomized prospective fashion. Moertel has shown 33.3% response rate with BCNU and 5-FU combined, as compared with a 16.1% response rate with 5-FU and 0% response with BCNU alone in advanced pancreatic carcinoma. All patients with histologically confirmed adenocarcinoma of the pancreas who are not amendable to curative surgery or primary radio-therapeutic management will be entered on the study.

Treatment regimen will consist of 5-Fluorouracil, 15 mg/kg per week, Mitomycin-C, 10 mg/m<sup>2</sup> intravenously every 6 weeks, Streptozotocin, 1 gm/m<sup>2</sup> intravenously once a week. To date, one patient has been entered.

## PATIENTS PLACED ON PROTOCOLS - 1 JANUARY - 31 DECEMBER 1975

## A. ACUTE LEUKEMIA COOPERATIVE GROUP B

I. Leukemia

<u>No.</u>	<u>Name</u>	<u>Diagnosis</u>	<u>Study</u>	<u>Date</u>
1.	Kieffer, Alder Jr.	A.L.L.	7411	13 Jan
2.	Lawrence, Keith	A.L.L.	7411	3 Jan
3.	Hines, Georgia	A.M.L.	7421	26 Jan
4.	Weeks, Tana	A.L.L.	7411	25 Feb
5.	Shupe, William	A.L.L.	7411	7 Mar
6.	Cauthem, Mary	A.M.L.	7421	21 Mar
7.	Little, Catharine	A.L.L.	7411	24 Mar
8.	Abreu, Linda	A.M.L.	7421	18 Mar
9.	Shorter, Waltraud	A.M.L.	7421	20 Mar
10.	Bussell, William	A.M.L.	7421	11 Apr
11.	Alfonso, Alexander	A.M.L.	7421	15 Apr
12.	Woods, Willard	A.M.L.	7421	8 May
13.	Alan, Joseph	A.L.L.	7411	9 May
14.	Evans, Alex	A.L.L.	7411	15 May

A. ACUTE LEUKEMIA COOPERATIVE GROUP B (Cont)

I. Leukemia (Cont'd)

<u>No.</u>	<u>Name</u>	<u>Diagnosis</u>	<u>Study</u>	<u>Date</u>
15.	Jameson, Christle	A.L.L.	7411	25 May
16.	McCallum, Bill	C.M.L.	7331	30 May
17.	McCall, Steven	A.L.L.	7113	30 Jun
18.	Grones, Dow	C.M.L.	7331	30 Jun
19.	Wall, Antoinette	C.M.L.	7331	22 Aug
20.	Dankow, Raymond	A.L.L.	7411	22 Aug
21.	Elert, Josef	A.M.L.	7521	4 Sep
22.	Blais, Edward	A.M.L.	7521	5. Sep
23.	Townsley, Clarence	A.M.O.L.	7521	20 Sep
24.	Mock, John	A.M.L.	7521	3 Oct
25.	Waldour, Gregory	A.L.L.	7411	22 Oct
26.	Smallwood, Keith	A.M.L.	7521	23 Oct
27.	Prioleau, Knowel	A.M.L.	7521	10 Jun
28.	O'Leary, Evelyn	Subacute M.L.	7521	2 Oct
29.	Henderson, Evelyn	A.M.L.	7521	20 Nov

## A. ACUTE LEUKEMIA COOPERATIVE GROUP B (Cont)

## I. Leukemia (Con't)

No.	Name	<u>Diagnosis</u>	<u>Study</u>	Date
30.	Parnell, Robert	A.M.L.	7521	21 Nov
31.	Cox, Rosalind	C.M.L.	7531	15 Dec
32.	Hassett, Frank	C.M.L.	7331	16 Dec

## II. Lymphoma

1.	Duke, Robert	Hodgkin's Disease	7251	30 Jun
2.	Calhoun, Susan	Hodgkin's Disease	7451	7 Aug
3.	Harrover, Charles	Hodgkin's Disease	7551	30 Oct
4.	Seitzer, Francis	Lymphoma	7253	13 Nov
5.	Arendas, William	Hodgkin's Disease	7552	4 Dec

A. ACUTE LEUKEMIA COOPERATIVE GROUP B (cont)

No.	Name	<u>Diagnosis</u>	<u>Study</u>	Date
1.	Christian, Godfrey	Multiple Myeloma	7361	31 Jan
2.	Bagley, Allie	Multiple Myeloma	7361	24 Jun
3.	Layton, Hannah	Multiple Myeloma	7361	11 Jul
4.	Gutierrez, Victor	Multiple Myeloma	7261	12 Dec
III. Multiple Myeloma				
IV. Solid Tumors				
1.	Fredericks, Frances	Small Cell Ca of Lung	7283	29 Jan
2.	Larson, Barbara	Ewing's Sarcoma	7391	25 Jan
3.	Kantrineitis, John	Oat Cell Ca of Lung	7283	5 Feb
4.	Persianni, Harry	Small Cell Ca of Lung	7283	7 Feb
5.	Fudold, John	Small Cell Ca of Lung	7283	20 Feb
6.	Johnson, Felix	Oat Cell Ca of Lung	7283	27 Feb
7.	Bowles, Walter	Small Cell Ca of Lung	7283	16 May
8.	Williams, J.C.	Oat Cell Ca of Lung	7283	2 Jul
9.	McLamarra, Mary	Ca of Breast	7581	15 Jul
10.	Johnson, Noland	Osteogenic Sarcoma	7181	17 Sep

A. ACUTE LEUKEMIA COOPERATIVE GROUP B (Cont)

IV. Solid Tumors (Cont)			
11.	Van Saders, Marie	Ca of Breast	7581 18 Sep
12.	Bitting, Winfield	Oat Cell Ca of Lung	7283 22 Sep
13.	Davis, William	Small Cell Ca of Lung	7283 10 Oct
14.	Martinson, Marcon	Ca of Breast	7581 2 Dec

B. WALTER REED ARMY MEDICAL CENTER PROTOCOLS

WRAMC #	Protocol	Diagnosis	Date
7205	Phase II Protocol Combination Chemotherapy with Dimethyl-Triazeno Imidazole Carboxamide (DIC) and Adriamycin in Soft Tissue and Bone Sarcomas		
1.	Mosley, Lloyd	Liposarcoma	10 Feb
2.	Willis, Benjamin	Liposarcoma	14 Feb
3.	Klerak, Genevieve	Metastatic Chordoma	23 Apr
4.	Anderson, Frances	Mesothelioma	20 May
5.	Herrity, Shane	Rhabdomyosarcoma	4 Aug
6.	Johnson, Sandra	Osteosarcoma	4 Aug
7.	Prestridge, Larry	Synovial Cell Sarcoma	4 Nov
8.	Pizzarro, Louis	Leiomyosarcoma	17 Nov

## B. WALTER REED ARMY MEDICAL CENTER PROTOCOLS (Cont)

WRAMC #7206 The Use of Methyl-CCNU (1-(2-Chloroethyl)-3-(4-Methylcyclohexyl)-1-Nitrosourea) (NSC 95441) in the Treatment of Brain Tumors

No.	Name	Diagnosis	Date
1.	Rodriguez, Mary	Glioblastoma Multiforme	11 Mar
2.	Morey, Vivian	Glioblastoma Multiforme	25 Apr
3.	Vance, Gloria	Glioblastoma multiforme	1 May
4.	Vonseth, William	Glioblastoma multiforme	3 Jul
5.	Eastwood, Marianne	Oat Cell Carcinoma	13 Jun
6.	Robison, Teresa	Astrocytoma	25 Jul
7.	Schwardt, Benjamin	Glioblastoma multiforme	27 Aug
8.	Green, Aurelia	Brain tumor	23 Aug
9.	Brown, Nolen	Oat Cell Carcinoma	16 Sep
10.	Hendorf, Earl	Glioblastoma multiforme	30 Sep
11.	Mayer, Elizabeth	Glioblastoma multiforme	28 Oct
12.	Anderson, Elwin	Glioblastoma multiforme	27 Jan
13.	Johnson, Diane	Astrocytoma II	21 Nov
14.	Kirks, Rowland	Glioblastoma multiforme	10 Dec
15.	Hauser, John	Astrocytoma II	30 Dec

B. WALTER REED ARMY MEDICAL CENTER PROTOCOLS (cont)

1975

WRAMC #7208 A Phase III Protocol - 5-Azacytidine in Acute Leukemia

- |    |                |     |        |
|----|----------------|-----|--------|
| 1. | Bell, Ronald   | CML | 16 May |
| 2. | Moods, Willard | AML | 8 Oct  |

WRAMC #7304 Study to Evaluate the Effect of Oophorectomy with and without Adjuvant Chemotherapy in the Management of Recurrent Mammary Carcinoma in Pre-Menopausal Women

- |    |                 |                          |        |
|----|-----------------|--------------------------|--------|
| 1. | Burns, Mildred  | Adenocarcinoma of breast | 31 Jan |
| 2. | Zeeks, Patricia | Metastatic breast ca     | 20 Jan |
| 3. | Waldron, Chung  | Metastatic breast ca     | 3 Jul  |

WRAMC #7401 Treatment of Advanced Lung Cancer with a Combination of Emetine (NSC-33669) and Cyclophosphamide or 1-(2-chlorethyl)-3-cyclohexyl-1-nitrosourea (CCNU) (NSC 79037)

- |    |                |                         |        |
|----|----------------|-------------------------|--------|
| 1. | Gordon, Joseph | Oat Cell Carcinoma      | 28 Mar |
|    |                |                         | 100    |
| 1. | Dillon, Clyde  | Embryonal Testicular ca | 19 Sep |

WRAMC #7402 Protocol for Adjuvant Therapy of Stage II Testicular Carcinoma with Chemotherapy (Actinomycin D and Chlorambucil), Radiation Therapy or Chemotherapy Plus Radiation Therapy After Retroperitoneal Lymph Node Dissection

- |    |               |                         |        |
|----|---------------|-------------------------|--------|
| 1. | Dillon, Clyde | Embryonal Testicular ca | 19 Sep |
|----|---------------|-------------------------|--------|

B. WALTER REED ARMY MEDICAL CENTER PROTOCOLS (Cont)

WRAMC #7403 Treatment of Advanced Lung Cancer with a Combination of 1,2-di(3,5-dioxyphiperazine-1-yl-propane) (ICRF-159) and Adriamycin with Cyclophosphamide Maintenance

1. Murphy, George Large Cell ca of the lung 19 Nov
2. Williams, J.C. Oat Cell carcinoma of the lung 8 Dec

WRAMC #7404 Immunological Evaluation and Immunotherapy of Patients with Carcinoma of the Lung

1. Laska, Leonard Squamous Cell Carcinoma of the lung 7 Jan
2. Altrasi, Andrew Squamous Cell Carcinoma of the lung 10 Jan
3. Ludden, John Squamous Cell Carcinoma of the lung 17 Jan
4. Carter, Joseph Large Cell Carcinoma of the lung 30 Jan
5. Faulconer, Dorothy Squamous Cell Carcinoma of the lung 4 Feb
6. Hicks, Shelby Adenocarcinoma of the lung 10 Feb
7. Baker, James Squamous Cell Carcinoma of the lung 25 Feb
8. Booram, Owen Epidermoid Carcinoma of the lung 9 Apr
9. Kennedy, John Squamous Cell Carcinoma of the lung 22 Apr
10. Witter, Robert Undiff. Large Cell Ca of the lung 8 Apr

B. WALTER REED ARMY MEDICAL CENTER PROTOCOLS (Cont)

1975

	<u>WPAMC #7404</u> (Cont)	
11.	Welch, William	Squamous Cell Carcinoma of the lung 2 Apr
12.	Carmony, John	Squamous Cell Carcinoma of the lung 20 Jan
13.	Wolin, Morris	Adenocarcinoma of the lung 22 Jun
14.	Leatsch, Arthur	Large Cell Carcinoma of the lung 1 Jul
15.	Williams, Clarence	Squamous Cell Carcinoma of the lung 10 Oct
16.	Hughes, Levi	Adenocarcinoma of the lung 29 Oct
17.	Olcisner, Carl	Epid. Ca of the lung 31 Oct

WPAMC #7405 Treatment of Advanced Penal Cell Carcinoma with a Combination of  
1-(Chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) (NSC 79037)  
and Bleomycin (NSC 125066)

1.	Baldino, Frank	Renal Celo Carcinoma 24 Feb
2.	Bilger, Thordis	Hypernephroma 24 Apr
3.	Slessman, Dale	Renal Adenocarcinoma 6 May
4.	Butcher, Charles	Hypernephroma 29 Jul
5.	Meyers, Rhona	Renal Cell Carcinoma 4 Nov
6.	Caldwell, Robert	Clear Cell Ca, Kidney 29 Aug

B. WALTER REED ARMY MEDICAL CENTER PROTOCOLS (Cont)

1975

WRAMC #7405 cont

Nichols, Sarah  
7.

Renal Cell Carcinoma 29 Oct

WRAMC #7406 Chemoimmunotherapy of Carcinoma of the Large Bowel

- |     |                   |                                      |        |
|-----|-------------------|--------------------------------------|--------|
| 1.  | Lynch, Margaret   | Adenocarcinoma of the Colon          | 10 Feb |
| 2.  | Biscoe, Leroy     | Adenocarcinoma of the Colon          | 27 Jan |
| 3.  | Lynch, Mildred    | Carcinoma of the Rectum              | 13 Jan |
| 4.  | Asai, Winnifred   | Carcinoma of the Colon               | 26 Jan |
| 5.  | Hileman, Betty    | Colon Adenocarcinoma                 | 22 Oct |
| 6.  | Slater, Isabel    | Dukes B, Carcinoma of the Colon      | 28 Oct |
| 7.  | Hansell, Olivia   | Adenocarcinoma of the Colon, Dukes B | 10 Nov |
| 8.  | Doheny, John      | Adenocarcinoma of the Colon          | 10 Nov |
| 9.  | Collins, James    | Carcinoma of the Colon               | 10 Nov |
| 10. | Roberts, Guy      | Stage III Colon Carcinoma, Dukes C   | 3 Dec  |
| 11. | Williams, Charles | Bowel Carcinoma, Dukes IIIC          | 29 Dec |
| 12. | Walters, Oliver   | Colon Carcinoma, Dukes C             | 29 Dec |

B. WALTER REED ARMY MEDICAL CENTER PROTOCOLS (Cont)

WRAMC #7407 Chemoimmunotherapy of malignant melanoma (Patterned after C.M.  
Anderson Protocols)

- |     |                     |                             |        |
|-----|---------------------|-----------------------------|--------|
| 1.  | Larche, Florent     | Malignant Melanoma          | 18 Jan |
| 2.  | Anderson, Rodger    | Malignant Melanoma          | 18 Feb |
| 3.  | Ellithorpe, Charles | Melanoma                    | 20 Feb |
| 4.  | Flurry, Paul        | Malignant Melanoma          | 3 Mar  |
| 5.  | Hodgin, Ralph       | Malignant Melanoma          | 18 Mar |
| 6.  | Johnson, Roy        | Malignant Melanoma          | 10 Jun |
| 7.  | Epps, Ferdinand     | Malignant Melanoma, Stage I | 7 Aug  |
| 8.  | Rives, Thomas       | Melanoma                    | 14 Oct |
| 9.  | Bache, Ben          | Melanoma, Stage I           | 3 Dec  |
| 10. | Crowley, Ellsworth  | Melanoma, Stage III         | 22 Dec |
| 11. | Sauter, Richard     | Melanoma, Stage III         | 22 Dec |

## B. WALTER REED ARMY MEDICAL CENTER PROTOCOLS (Cont)

1975

WRAMC #7408 Comparative Trial of Tamoxifen and Fluoxymesterone Plus Tamoxifen in Metastatic Breast Cancer

- |    |                   |           |        |
|----|-------------------|-----------|--------|
| 1. | Wilson, Patricia  | Breast Ca | 6 Mar  |
| 2. | Costello, Eleanor | Breast Ca | 19 May |
- WRAMC #7410 Combination of NeCCNU Plus VP 16-213 in the Treatment of Metastatic Adenocarcinoma of the Gastrointestinal Tract and the Pancreas
- |    |                 |                             |        |
|----|-----------------|-----------------------------|--------|
| 1. | Burd, Henry     | Adenocarcinoma of the colon | 24 Jan |
| 2. | McCarthy, James | Adenocarcinoma of the colon | 26 Feb |

WRAMC #7412 Metastatic Breast Ca Study to Evaluate the Effect of Cyclophosphamide, Adriamycin and 5-FU vs. Adriamycin, Dibromodulcitol and Vincristine Sequentially Alternating with Cyclophosphamide, Methotrexate and 5-FU

- |    |                 |                             |        |
|----|-----------------|-----------------------------|--------|
| 1. | Mellon, Jan     | Metastatic Breast Carcinoma | 10 Feb |
| 2. | Zeeks, Patricia | Metastatic Breast Carcinoma | 5 May  |
| 3. | Nickel, Julia   | Metastatic Breast Carcinoma | 25 Jun |
| 4. | Waldrum, Chung  | Metastatic Breast Carcinoma | 8 Oct  |

B. WALTER REED ARMY MEDICAL CENTER PROTOCOLS (Cont)

WRAMC #7501 Evaluation of Adriamycin and Cis-Platinum Combination Chemotherapy  
in Treatment of Malignant Disease

1. Looney, Jack Ca Prostate, Metastatic 18 Nov
2. Lyons, Leo Squamous Cell Ca of the esophagus 21 Nov
3. Lavery, David Transitional Ca, bladder 15 Oct
4. Price, Gelroy Adenocarcinoma of the lung 23 Oct

WRAMC #7502 A Pre-Test Trial of 2,5-Piperazinedione, 3,6-Bis-(5-Chloro-2-Piperidyl)-  
Dihydrochloride in the Treatment of Advanced Renal Cell Carcinoma

1. Irving, Imogene Renal Cell ca 17 Oct
2. Caldwell, Robert Renal Cell ca 28 Nov

WRAMC #7601 The Treatment of Unresectable Bronchogenic Carcinoma with CCNU  
(1-(2-Chloreethyl)-3-Cyclohexyl-1-Nitrosourea) (NSC 79037),  
Cyclophosphamide, Procarbazine, and Hexamethyl-melamine  
(NSC 13875)

1. Sandy, Kenneth Large Cell Undiff. Lung ca 22 Dec

Work Unit No.: 1901

Title of Project: The Efficacy of Antisera to Gram Negative Endotoxin  
in the Treatment of Gram Negative Sepsis

Investigators:

Principal: John L. Carpenter, MD

Associate: Jerald Sadoff, MD

Objective: To evaluate the efficacy of antisera which is made against  
a "common antigen" in the core of the endotoxin of gram negative  
rods in treating suspected or documented gram negative sepsis.

Technical Approach: Patients with documented or suspected gram negative  
sepsis were given antisera in addition to standard antibiotic and  
supportive therapy. The antisera was administered in a double  
blind fashion in that two units had been prepared from each donor;  
one obtained pre-immunization and one post-immunization. An in-  
dividual patient would receive either the pre or post-immunization  
sera. The patients were clinically evaluated pre and post therapy  
by the investigators and the data recorded on standard flow sheets.  
This clinical information was then relayed to Dr. Elizabeth Ziegler  
at the University of California at San Diego, who is coordinating  
this multi-center study.

Progress and Results: During FY 76, 10 units of antisera were given with-  
out significant complications. The efficacy of the antisera has not  
been determined as the code in this double blind study has not yet  
been broken.

Conclusions: Deferred at present.

Funds Utilized, FY 76: None.

Funding Requirements, FY 77: None.

Publications: None.

Type of Report: Interim - Annual Progress Report.

Work Unit No.: 1902

Title of Project: The Use of Antibiotic and Antiseptic Ointments with Intravenous Catheters

Investigators:

Principal: John L. Carpenter, MD

Associate: Robert Enquist, MD

Objective: To determine the efficacy of applying antibiotic or antiseptic ointment to the site of intravenous catheterization.

Technical Approach: Patients receiving intravenous catheters in the ICU-CCU were randomized to have either Betadine®, Neosporin G®, or no ointment placed at the site of intravenous catheterization. The intravenous dressings and ointments were changed daily and the site of catheterization inspected for phlebitis. When the catheter was removed it was cultured in trypticase soy (TSB) broth. The patients were also observed for the development of sepsis secondary to the catheter. The three groups were then analyzed as per incidence of phlebitis, positive catheter cultures and the development of catheter-induced sepsis. The effect of parenteral antibiotics, steroids, heparin and lidocaine on the above parameters was also determined.

Progress and Results: Eighty-five patients were entered into the study in FY 76. To date there appears to be no significant difference between the three groups as per the above parameters. Neither parenteral antibiotics, steroids, heparin or lidocaine affected the parameters being evaluated.

Conclusions: Deferred as study not complete.

Funds Utilized, FY 76: Obtained from OMA funds thru Department of Bacterial Diseases, WRAIR.

Funding Requirements; FY 77: See above.

Publications: Abstract - International Conference on Antibiotics and Chemotherapy (ICAAAC) - abstract has been submitted.

Type of Report: Annual Progress Report - interim report.

Work Unit No.: 2004

Title of Project: Protein Sparing Effects of 3.5% Fibrin Hydrolysate vs 5% Dextrose in Surgical Patients

Investigators:

Principal: Mitchell V. Kaminski, LTC, MC

Associate: Richard D. DeShazo, CPT, MC; Dawn E. Carlson, CPT, MSC; Wayne Wilson, LTC, MC; and Jerry Earll, COL, MC

Objective:

Can the infusion of 3.5% solutions of fibrinohydrolysate (Aminosol<sup>R</sup>, Abbott Laboratories) produce positive nitrogen balance in post-operative surgical patients?

Technical Approach:

See detail sheets inclosed.

Progress and Results:

See detail sheets inclosed.

Conclusions:

See detail sheets inclosed.

Funds Utilized, FY-76:

None

Funding Requested, FY-77:

None

Publications:

1. Kaminski, M., N. Dunn, R. Wannemacher, R. Dinterman, R. DeShazo, W. Wilson, J. Earll, D. Carlson. Mechanism for protein sparing during postoperative dextrose free amino acid infusions. Clinical Research XXIV:3:501A, 1976.

2. Kaminski, M.V., N.P. Dunn, R.W. Wannemacher, R.E. Dinterman, R. DeShazo, W.W. Wilson, D. Carlson. Specific muscle protein-sparing postoperative dextrose-free amino acid infusions. Submitted for publication, American Journal of Clinical Nutrition.

Type of Report:

Terminated

Presentation, American Society for Clinical Nutrition,  
Atlantic City, N. J., 1 May 1976, CLINICAL RESEARCH  
XXIV:3:501A, April 1976

MECHANISM FOR PROTEIN SPARING DURING POSTOPERATIVE DEXTROSE FREE AMINO ACID INFUSIONS. M. Kaminski\*, N. Dunn\*, R. Vanacekcher, R. Dinterman\*, R. DeShazo\*, W. Wilson\*, J. Earlt\*, D. Carlson\*. USAFRMD, Fort Detrick, Frederick, MD and NAMC, Washington, DC.

In an effort to improve nutritional support of injured patients an hypothesis was tested which held that intravenous infusions of dextrose free amino acid (AA) solutions could induce a change in hormone profile which simultaneously promotes protein sparing and beneficial fat catabolism. To accomplish this 31 patients with normal hepatic and pancreatic function scheduled for similarly traumatic elective abdominal surgery were randomly assigned to receive a postoperative maintenance infusion of either 3.5% AA (fibrinhydrolylate) or 5% dextrose in 1/3 normal saline. This postoperative period of study lasted 3 days, ending when oral nutrition was resumed. Blood was drawn between 1100 and 1200; 24-hr urine collections were made daily. No significant differences were observed in serum 17-hydroxycorticosteroids and urine 17-hydroxycorticosteroids, VMA and metanephrine in the two groups. In the AA group, serum glucagon and growth hormone activity, as evidenced by urinary hydroxyproline excretion, were significantly increased. Serum insulin was slightly but not significantly reduced; serum glucose was lower; and ketoaciduria was present in all subjects. Urinary 3-methylbiscidine and nitrogen balance were improved which corroborated a protein-sparing effect. No patient in the dextrose group showed ketoaciduria.

It is concluded that an infusion of the AA solutions at these concentrations did not stimulate insulin release but elevated serum glucagon concentration and growth hormone activity. This resulted in increased lipolysis, ketogenesis and sparing of endogenous protein.

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Work Unit No.: 2101

Title of Project: Investigation of Vascular Injuries, Vascular Disease, Vascular Grafts, and Operating Procedures

Progress and Results: Effort continues in the Peripheral Surgery Service and Clinic to determine the long term fate of various types of vascular reconstructions. We continue to study the natural progression of both disease and injury to blood vessels. We are entering an exciting period in our to ten year follow-up of the casualties sustaining vascular injuries in Vietnam. This remains an important mission for the military in our continuing attempt to identify the best conduit for blood vessel repair to save lives and salvage limbs, as well as the ability to return casualties to full active duty. Because Vascular Surgery remains a relatively new field, these repeated evaluations in a long term follow-up are paramount. The information that we are accumulating is the largest collection of vascular injuries ever documented. This material also provides valuable information for Allied Foreign Military groups and for our civilian counterparts in the United States. As far as the disease registry and follow-up are concerned, we continue to learn more about various types of repairs that will be of value to us if it becomes necessary to again perform them on the battlefield.

Our registry contains copies of medical records of nearly 7,500 casualties from Vietnam, approximately 300 Korean casualties, a scattered number of World War II casualties, and 10,000 patients with vascular disease. This registry is maintained and continually expanded. Patients are encouraged to return for routine follow-up visits. If this is not possible, questionnaires are sent by mail and assistance of other doctors from both military and civilian hospitals is solicited. We are continuing excellent exchange with the research effort of Walter Reed Army Institute Res-arch (WRAIR) at Walter Reed Army Medical Center (WRAMC), the Cardiovascular Branch, of Armed Forces Institute of Pathology (AFIP), the Bio-Physical Laboratory Edgewood Arsenal, Maryland, and George Washington University School of Medicine.

Progress continues similar to previous years reflected by an outstanding quality of patient care with the accomplishment documented for both the clinical and research vascular projects as outlined in the bibliography. Numerous presentations have reflected positive reactions. Recognitions of this work in the academic community has been demonstrated by Dr. Rich being selected for two important committees, Committee on Truma and the Committee for International Relations in the American College of Surgeons, election into membership of the 250 member Society of University of Surgeons, and election into the International Surgical Society. Dr. Rich has also worked with the Committee of Government Experts under the auspices of the International Committee of the Red Cross to determine effects of wounding and subsequent management of combat casualties. Dr. Rich, was invited to be the faculty opponent against a Swedish surgeon on his PhD thesis. Numerous, similar exchanges have existed with other foreign organizations and this has helped establish positive thinking toward our Army Medical Department. The second meeting of Military Vascular Surgeons and the Second Annual WRAMC Vascular Seminar were an outstanding success last December. With the Vascular fellowship unique in the Army, and one of the oldest in existence approaching ten years of continuous activity, we are in the position to provide leadership in vascular surgery. It is also important for us to insure that all who have taken the Vascular fellowship remain in a strong position for continuing education.

Work Unit No.: 2102

Title of Project: Effects of Operative Stress on the Coagulation Profile

Investigators:

Principal: LTC George J. Collins, Jr., M.D., MC

Associate: MAJ David J. Ahr, M.D., MC  
COL Norman M. Rich, M.D., MC  
LTC Robert W. Hobson, M.D., MC  
MAJ Charles A. Andersen, M.D., MC

Objectives: To determine to what degree various degrees of surgical stress affect the coagulation profile and to determine whether or not patients with high risks of thrombosis can be identified by laboratory testing.

Technical Approach: The following laboratory tests have been evaluated to determine their usefulness in confirming the clinical impression of hypercoagulability: hematocrit, prothrombin time, activated partial thromboplastin time, platelet count, fibrinogen, anti-thrombin III, thrombin time, platelet factor III availability, platelet aggregation, factors II, V, VII, VIII, IX, XI, XII, fibrin degradation products, and fibrin monomer.

Progress and Results: A great deal of progress in this area has been made in the last year. So far, we have studied approximately 60 patients, and have hard data on 47 patients. Some patients had to be eliminated because it was found that they were taking drugs that interfered with the test results. It has been found, that we can confirm the clinical diagnosis of hypercoagulability with an accuracy approaching 90%. We have also found that some of the tests are of no value in predicting hypercoagulability while some of the tests simply duplicate information gained from other testing procedures. Having ascertained this, we have been able to eliminate some tests and now have arrived at a condensed coagulation profile involving only six tests. Even so, we have not diminished the diagnostic accuracy and have effected the saving of a great deal of technician time.

Conclusions: The twofold objective of this protocol has been achieved. The objective of determining to what degree various degrees of surgical stress affected the coagulation profile has been satisfied by previous research projects. Tabulation of that data indicated that there is a trend toward hypercoagulability in the postoperative period which is manifested around the third postoperative day and continues to the seventh postoperative day. The second objective which was to determine whether or not patients with high risks of thrombosis could be identified by laboratory means has been satisfied. To repeat, we believe that we can reliably detect hypercoagulability with an accuracy approaching 90%.

Funds Utilized, FY-76: Up to this date \$1421.85 has been used to purchase medical supplies. We now have a balance of \$978.15 and it is anticipated

that these funds will be needed to purchase medical supplies also.

Funding Requirements, FY-77: No funds will be required because it is anticipated that this project will be terminated as complete prior to the beginning of FY-77.

Publications: During FY-76 the senior investigator attended the South-western Surgical Congress in Houston, Texas and presented a paper entitled, "Detection and Management of Hypercoagulability", which has been submitted for publication in the American Journal of Surgery. In addition, an exhibit entitled, "Hypercoagulability - Importance in Surgical Practice", has been accepted for display at the American Medical Association Meeting in Dallas, Texas in June 1976.

Type of Report: Completed.

Work Unit No.: 2103

Title of Project: Heparin Dosage during Peripheral Vascular Reconstruction

Investigators:

Principal: LTC George J. Collins, Jr.

Associate: MAJ David J. Ahr  
MAJ William Armstrong  
COL Norman M. Rich  
MAJ Charles A. Andersen

Objective: To determine the safe and effective dose of heparin for use during peripheral vascular reconstructive procedures.

Technical Approach: Twenty-two patients undergoing peripheral vascular reconstructive procedures for lower extremity ischemia or abdominal aortic aneurysm were randomly assigned to two groups to receive either 100 or 150 units/kgm. of body weight (u/kgm.) of heparin intravenously just prior to cross clamping. The thrombin clotting time assay was used to monitor heparin levels. Assays were done prior to skin incision, just prior to heparinization, 5 minutes after heparinization and at each 15 minute interval thereafter, just prior to heparin reversal with protamine sulfate, 5 minutes after reversal, and at the time of skin closure.

Progress & Results: The levels of heparin achieved 5 minutes after heparinization were  $2.23 \pm 0.61$  units/ml. (u/ml.) plasma (mean  $\pm$  S.E.M.) in the group receiving 100 u/kgm. and  $2.60 \pm 0.22$  u/kgm. in the group receiving 150 u/kgm. These levels were not significantly different from one another ( $p > 0.05$ ). However, at all subsequent intervals, the amount of heparin remaining was significantly higher in the group receiving 150 u/kgm. Twenty minutes after heparinization, the heparin level was  $1.55 \pm 0.20$  u/ml. in the group receiving 100 u/kgm. and  $2.14 \pm 0.18$  u/ml. in the group receiving 150 u/kgm. ( $p < 0.05$ ). After 35 minutes, heparin levels were  $0.88 \pm 0.12$  u/ml. and  $2.03 \pm 0.41$  u/ml. in the groups receiving 100 u/kg. and 150 u/kg., respectively ( $p < 0.05$ ). After 50 minutes, heparin levels had dropped to  $0.85 \pm 0.09$  u/ml. and  $1.73 \pm 0.25$  u/ml. in the groups receiving 100 u/kg. and 150 u/kg., respectively ( $p < 0.005$ ). In patients with clamp times longer than 65 minutes, the heparin level was  $0.71 \pm 0.31$  u/ml. in the group receiving 100 u/kgm. and  $1.78 \pm 0.16$  u/ml. in the group receiving 150 u/kgm. ( $p < 0.001$ ).

All but one patient still had therapeutic levels (0.2-0.5 u/ml.) of heparin circulating just prior to reversal with protamine. Eighteen percent of patients failed to return to less than therapeutic levels of heparin using 0.5 mg. of protamine per 100 units of heparin administered. One patient with massive heparin consumption developed severe thrombotic complications.

Conclusions: These studies indicate that there is an initial peak level of heparin after intravenous injection followed by sequential diminution of

circulating levels. Disappearance rates of heparin from plasma appear to be dose related and non-linear. These studies also indicate the necessity for heparin reversal even with moderate initial doses. Furthermore, they suggest that laboratory evidence of massive heparin consumption may be premonitory evidence of massive thrombosis. Further studies are necessary to confirm or refute these preliminary observations.

Funds Utilized, FY-76: \$843.20 was used for medical supplies.

Funding Requirements, FY-77: \$1,000.

Publications: None.

Type of Report: Interim.

Work Unit No.: 2204

Title of Project: Causalgia: A study of sympathetic activity in affected patients.

Investigators:

Principal: LTC Albert J. Tahmoush, M.D.

Associate: CPT John R. Jennings, Ph.D., Frederick W. Hegge, Ph.D., LTC Albert N. Martins, M.D.

Objectives: To determine if abnormalities in sympathetic activity are consistently associated with causalgia.

Technical Approach: Indirect estimates of local sympathetic nervous system activity were obtained through measurements of skin conductance (SC), cutaneous blood volume (BV) and blood volume pulse amplitude (BVP). Skin conductance was determined by the constant voltage method. BV and BVP were obtained with a photoelectric plethysmograph developed for this study. Measurements were performed in a controlled environmental chamber on two consecutive days in two 30-minute periods following an AB-BA design. For each measure, the affected and non-affected extremities were compared.

Progress and Results: Nine patients with causalgia of at least 18 months duration and nine age and sex-matched normal subjects were studied. Asymmetries between affected and non-affected extremities for the three measures were seen in patients and controls. However, the asymmetries were not consistent across subjects. Analysis of variance revealed no statistically significant asymmetry in skin conductance, cutaneous blood volume or blood volume pulse amplitude in the patient or control group. An analysis of variance within measure and between groups also revealed no statistically significant difference.

Conclusions: The results of this study suggest that increased sympathetic nervous system activity is not consistently associated with causalgia and probably does not serve as mediator of this pain syndrome. Our results suggest that the term "reflex sympathetic dystrophy" should not be applied to causalgia.

Funding requirements: None

Publications: Manuscript is in preparation

Type of Report: Interim

Work Unit No.: 2206

Title of Project: Chymopapain in Treatment of Lumbosacral Intervertebral Disc Disease

Investigators:

Principal: Albert N. Martins, COL, MC

Associate: Archimedes Ramirez, LTC, MC - Paul R. Schwetschenau, MAJ, MC

Objectives: To evaluate the safety and efficacy of intradiscal injection for treatment of lumbar intervertebral disc disease and to evaluate the efficacy of the drug Chymopapain for intradiscal injection by means of a double-blind study utilizing a placebo.

Technical Approach: Sixty-six patients with signs, symptoms and a myelographic abnormality of herniated lumbar disc, not responsive to conservative treatment, were injected intradiscally at random with either Chymopapain or a placebo. Neither patient nor surgeon knew which agent was used until after the results had been tabulated.

Progress and Results: Unless early laminectomy was necessary for intractable pain, all patients were followed for 2 months or more. There was no statistically significant difference in incidence or quality of improvement between the 2 groups: Chymopapain was successful in 58% while placebo was successful in 49% ( $p=.15$ ).

Conclusions: Early results indicate that most, if not all, of the putative effectiveness of chemonucleolysis probably derives from a placebo effect.

Funds Utilized FY-76: None

Funds Requested FY-77: None

Publications: None in FY-76 (Submitted for Publication J. Neurosurgery)

Type of Report: Completed

Work Unit No.: 2302

Title of Project: Study of the Use of Radioactive Phosphorous in the Diagnosis of Melanoma Tumors of the Eye

Investigators:

Principal: Paul V. Whitmore, MD, LTC, MC, Ophthalmology Service, WRAMC

Associate: Kenyon K. Kramer, MD, LTC, MC, Ophthalmology Service, WRAMC

Objectives: The objective of this research is to determine the effectiveness of radioactive phosphorous (P-32) in the diagnosis of intraocular tumors.

Technical Approach: Patients with suspected intraocular tumor will be evaluated using radioactive P-32 and a solid state beta detector following the method of Hagler. The test results will then be compared with either the subsequent clinical course in cases where enucleation is not done or with the histopathological findings in cases where enucleation is done.

Progress & Results: To date, 20 patients have been examined with the P-32 test. Eleven of these patients had choroidal or subretinal masses and 9 had iris or ciliary body masses. The P-32 test was positive in 8 out of 8 histologically proven malignant choroidal melanomas and was negative in 2 out of 3 benign choroidal masses. The single false negative study was obtained in a patient who had a large benign melanocytoma which harbored a small focus of malignant transformation.

While the test was accurate in the diagnosis of choroidal or subretinal masses, it was not as accurate where iris or ciliary body masses were studied. Of the 9 cases so studied, one had a positive test and was histologically proven to be a melanoma. Of the remaining 8 cases which had negative P-32 studies, 2 underwent change and were subsequently histologically proven melanomas. The remaining 6 cases have been clinically unchanged, following a benign course.

Conclusions: The P-32 test is a clinically important adjunctive test in aiding the diagnosis of choroidal melanomas. It is less accurate and should not be used solely for the diagnosis of iris lesions, which should best be managed on the basis of documented growth. We will continue to apply this test as a diagnostic aid in cases of suspected choroidal melanoma.

Funding Requirements: None

Personnel: None

Publications: "32 P Test On Uveal Tumors" presented at the 6th Biennial  
Walter Reed Ophthalmology Postgraduate Course - 20 April 1976

Type of Report: Completed

Work Unit No.: #2303

Title Of Project: "Inquiries into Certain Biochemical and Ultra-structural Features of Pediatric Corneal Tissue"

Investigators: Major George R. Beauchamp

Associate: None

Objectives: N/A

Technical Approach: N/A

Progress & Results: Dr. Beauchamp has separated from the service without having any progress to report.

Conclusions: N/A

Funding Requirements: We cannot support this project.

Work Unit No.: 2304

Title of Project: Technician-Assisted and Computer-Assisted Ophthalmological Care

Investigators:

Principal: COL Budd Appleton

Associate: LTC Kenyon K. Kramer

- Objectives:
- (1) To evaluate current methods of refractometry in terms of which type and methods appear to have the greatest military medical application.
  - (2) To evaluate the potential of civilian-trained Ophthalmic Medical Assistants (Technicians) for integration as members of the Army Eye-care Team.
  - (3) To evaluate methods of training Ophthalmology residents so as to include maximum utilization of allied health personnel in their training for clinical practice.
  - (4) To determine the optimum composition of a Program of Instruction (POI) for Ophthalmic Medical Assistants trained to work in AMEDD MTF's.

Technical Approach:

- (1) Comparison studies of time required to do manual refractometry (retinoscopy vs the time required to use clinically available automated retinoscopes (Auto-refractor 6600, Ophthalmetron, and Dioptron).
- (2) Measurement of increase in rate of patient throughput per ophthalmologist by using a civilian-trained Ophthalmic Technician to perform selected psychomotor tasks ordinarily performed by the doctor. Measurements to be made using several samples of ophthalmologists and several samples of technicians, plus whatever variations in task arrays and order appear to be appropriate. Measurements to be obtained using two or more Ophthalmic Technicians working simultaneously with the same ophthalmologist if this is feasible.

(3) Establishment of proposed SOP's for:

- a. Use of one or more Ophthalmic Technicians by an ophthalmologist in an AMEDD MTF Eye Clinic.
- b. A Program of Instruction for Army-trained Ophthalmic Medical Assistants trained specifically to do work in AMEDD MTS's.
- c. Integration of training in utilization of Ophthalmic Medical Assistants into the P01's for ophthalmology residents trained in AMEDD MTF's.

**Progress & Results:** Project has been under way nine (9) months, of which approximately two (2) months were required for task partition and familiarization with clinical procedures. All of the remaining time has been devoted exclusively to Objective No.(2) and its Technical Approach. The performance levels of two (2) residents have been incompletely evaluated as of this date. One resident (MAJ Hymarsh) showed a maximum increase of only 50% over base-line, while the other (MAJ Leedy) showed an increase of over 130%. Details of this data are contained in the bi-monthly Interim Progress Reports (Q.V. attached.) At present, a third resident (MAJ Wertz) has been on the project approximately two (2) weeks and his participation is not yet reflected in an Interim Progress Report, but in just this period he has shown an increase of over 140% over base-line.

**Conclusions:** As far as the investigators are concerned, contractor has been fulfilling the terms of his contract, and the project has been conducted to the maximum extent that integration with the clinical activities of this Service will allow. Investigators believe that progress has been quite satisfactory, and it is recommended that the project be continued with maximum emphasis on objective (2) until a sufficient number of doctors have been observed so that a reasonable average performance level can be predicted for ophthalmologists working in AMEDD MTF's.

**Funding:** Funding was accomplished through contract with the Educational Study Association of St. Paul, Minnesota, in association with the University of Minnesota Medical School, at a price of \$14,150. Under this one year contract the services of 2-year school-trained Ophthalmic Technician have been provided by ESA, starting 25 July 75. ESA is willing to renew this contract for an additional year, but because of increased costs (primarily salaries) they would like the price to be changed to \$18,500. This funding appears reasonable, and has been coordinated directly with HQ, USAM R&D Command (who will notify WRAMC Comptroller regarding exact funding data).

**Publications:** N/A

**Type of Report:** Interim Annual Progress Report

Work Unit No.: 2306

Title of Project: Clinical Quantification of Intraocular  
Malignant Melanoma Volume

Investigators:

Principal: Kenyon K. Kramer, LTC, MC, USA

Objectives: To develop a technique to quantitate the size of intraocular malignant melanomas *in vivo*, since this is an important prognostic parameter.

Technical Approach: B Scan water-bath ultrasonography after the method of Jackson Coleman, M.D., will be used to measure malignant melanomas *in vivo*, both volume and single largest dimension

Progress & Results: Four lesions have been measured *in vivo* and come to histopathology with the following results:

<u>Vol % Error</u>	<u>Single Largest Dimension % Error</u>
#1 -14%	+11%
#2 +80%	+71%
#3 +40%	+22%
#4 +84%	+45%

Conclusions: The B Scan technique gives only rough estimates, so far. The numbers are too small to detect a systematic variation, if present. Minor adjustments in technique may yield large improvements in accuracy.

Funds Utilized, FY-76: \$0

Funding Requirements, FY-77: \$0

Publications: None

Type of Report: Interim

Work Unit No.: 2501

Title: An Aerodynamic Evaluation of the Speech of Patients with Voice Disorders

Investigators:

Principal: Robert A. Prosek, CPT, MSC

Associate: Brian E. Walden, CPT, MSC  
Allen A. Montgomery, Ph.D.

Objectives: To determine how the breath stream is controlled by patients with voice disorders, and to determine if the aerodynamic parameters of speech may be used to distinguish among groups of patients having different voice disorders.

Technical Approach: Measurements of intraoral air pressure, air flow rate, volume of expired air, fundamental vocal frequency, sound pressure level and consonant duration are made during the production of words and sentences, and during a psychophysical scaling task. During this latter task, the patient is asked to systematically vary the vocal effort used to produce the consonants /p, b, s, z/.

Progress and Results: Three patients with vocal nodules, one patient with contact ulcers of the vocal folds, one patient with unilateral vocal fold paralysis, and one patient with bilateral vocal polyps have been tested. This is not a sufficient number of patients to perform statistical analyses of the data (multiple regression and discriminant analysis) or to draw valid conclusions. However, the data do show a number of encouraging trends.

The data gathered during the production of words and sentences reveal that intraoral air pressure, consonant duration and fundamental vocal frequency are parameters which may be potentially useful in separating patients on the basis of physical measurements of their speech. As shown in Table 1, patients with vocal nodules displayed high oral air pressure and fundamental frequency but low consonant durations. The patient with contact ulcers showed low oral pressures and fundamental frequency but high consonant durations. The patient with unilateral vocal fold paralysis demonstrated high oral air pressures but low consonant durations and fundamental frequency. Finally, the patient with bilateral polyps showed high air pressures, consonant durations and fundamental frequency.

When air flow rate, volume of expired air, fundamental vocal frequency, sound pressure level, and consonant duration were plotted on logarithmic coordinates as a function of vocal effort, no differences could be observed. However, as shown in Figure 1, when intraoral air

pressure is plotted as a function of vocal effort, two differences can be seen. First, the slope relating intraoral pressure to vocal effort is different for each type of patient. The slope for the polyps patient is .24; for the vocal nodules patient it is .3; for the contact ulcer patient, the slope is .4; and for the patient with unilateral paralysis, the slope is .5. In addition, it can be seen that the magnitude of pressure for each effort level ranks the patients, with the polyps patient having the highest pressures and the patient with contact ulcers consistently having the lowest pressures.

**Conclusions:** Because of the low number of patients that have been tested to date, no firm conclusions can be drawn from the data. However, the data do indicate that intraoral air pressure, consonant duration and fundamental vocal frequency have the potential for grouping the patients according to their speech patterns. These parameters also may be useful as training devices in therapy.

**Funds Utilized, FY-76:** None

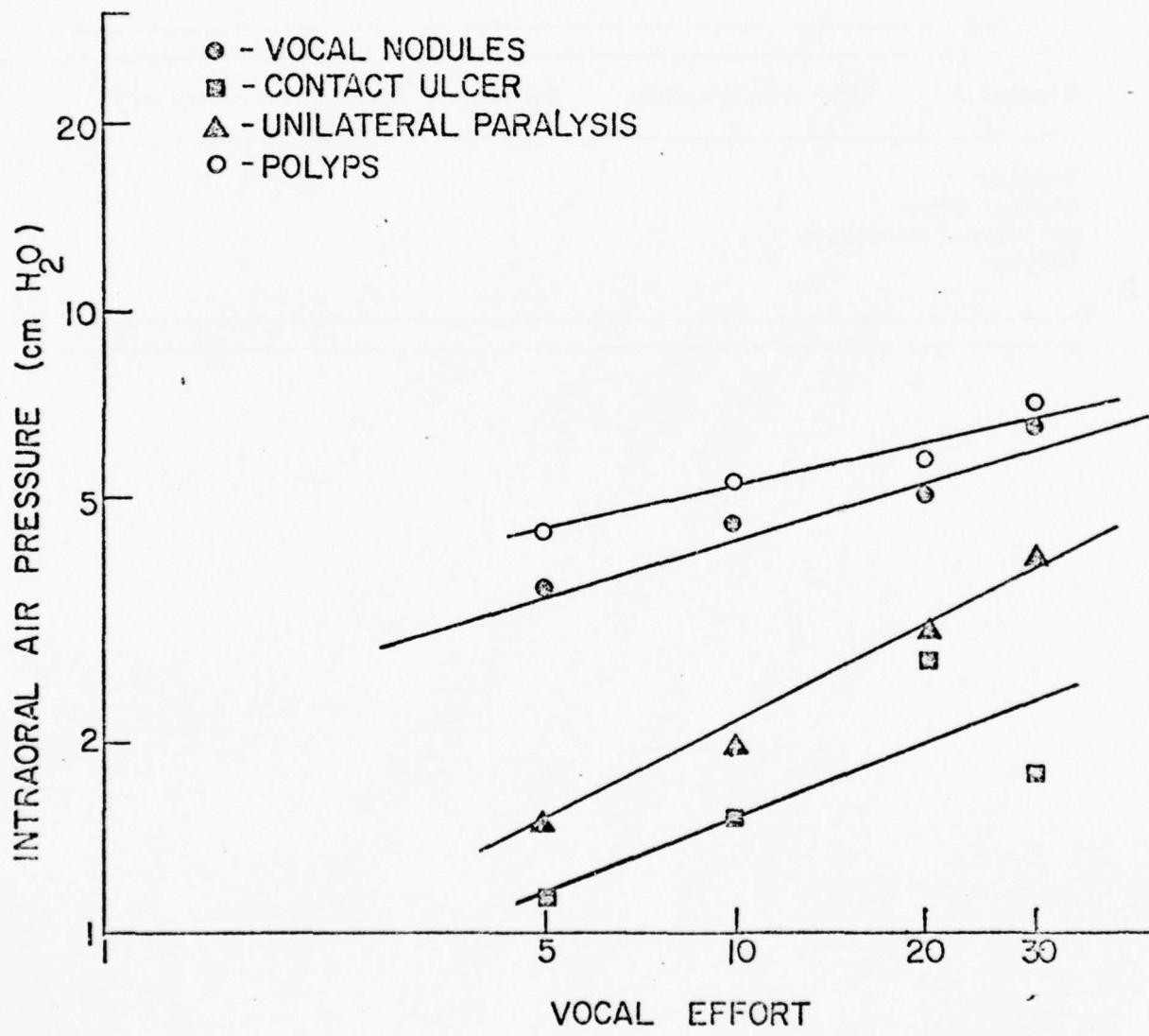
**Funding Requirements, FY-77:** None

**Publications:** Not Applicable

**Type of Report:** Interim

Table 1. Relative level of intraoral air pressure, consonant duration, and fundamental vocal frequency for the four types of voice patients. A plus sign indicates a high level of the parameter relative to the other patients; a minus sign indicates a low level of the parameter relative to the other patients.

Diagnosis	Oral Air Pressure	Duration	Fundamental Frequency
Nodules	+	-	+
Contact Ulcer	-	+	-
Unilateral Paralysis	+	-	-
Polyps	+	+	+



WORK UNIT NO: 2506

Title of Project: A Comparison of an Objective Hearing Aid Evaluation  
with a Subjective Hearing Aid Selection Procedure.

Principal Investigator: Rodney M. Atack, MAJ MSC

Work on the above numbered project was terminated 23 October 1975, because  
the project is not feasible in its present form. No funds were requested,  
allocated or expended.

Work Unit No: 2508

Title of Project: An Experimental Analysis of Aural Rehabilitation Using Programmed Instructions.

Investigators: Edward B. Muth, M.A.  
Supervisor  
Aural Rehabilitation Section

Charlene K. Scherr, M.A.  
Aural Rehabilitation Section

Objectives: To Provide 3 Programmed Presentations for Orientation to Effective Hearing Aid Use.

Technical Approach: Use of recorded 3-voice narration. 2 x 2 35 mm slides for each of 3 Sound Slide Projector Systems.

Progress and Results: The first of the 3 programmed presentations is now completed and in use at this Center. This programmed instruction in hearing aid use and adjustment is available for viewing to all hearing impaired persons wearing a hearing aid for the first time. The slides and tape will be duplicated for the other two presentations.

Conclusion: The project indicates that programmed presentations of this type are beneficial to hearing impaired military personnel in the often difficult adjustment to effective hearing aid use, especially in the absence of a complete Aural Rehabilitation Program.

Fund Requirements: N/A - No Funding.

Work Unit No.: 2509

Title of Project: Dimensions of Visual Consonant Perception in Hearing-Impaired Adults

Investigators:

Principal: Brian E. Walden, CPT, MSC  
Associates: Robert A. Prosek, CPT, MSC  
Allen A. Montgomery, Ph.D.

Objective: To determine the maximum number of visually contrastive (homophenous) consonant categories used by hearing-impaired adults in lipreading.

Technical Approach: See Procedures Section of attached manuscript.

Progress: All phases of this project have been successfully completed. The attached manuscript is currently under review by the Journal of Speech and Hearing Research.

Conclusions: The following major conclusions are justified based upon the data:

1. Lipreading training caused an increase in the number of homophenous consonant categories consistently recognized by hearing-impaired observers. Specifically, the number of visemes increased from five for the pre-training results to nine for the post-training results (75% within-category response rate).
2. Lipreading training caused an increase in the percentage of within-viseme responses.
3. Most of the changes in consonant recognition occurred during the first few hours of training.

Funding Requirements: None.

Publications: The attached manuscript is under review for publication in the Journal of Speech and Hearing Research.

Work Unit No.: 2510

Title of Project: A Multidimensional Assessment of Stuttering Severity

Investigators:

Principal: Robert A. Prosek, CPT, MSC

Associate: Brian E. Walden, CPT, MSC  
Allen A. Montgomery, Ph.D.

Objectives: To determine the parameters of stuttered speech that are used by speech pathologists to rate the severity of stuttering.

Technical Approach: Measurements of fundamental vocal frequency, intensity, pause time, word and phrase repetitions, prolongations, and rate of speech are made from the tape-recorded speech of ten stutterers. Various multivariate statistics are used to determine which parameters are the most important in characterizing the stuttered speech. The speech samples are paired and played for a group of ten speech pathologists who are asked to rate the similarity of the stuttering patterns on a seven-point scale. These similarity data are analyzed by means of a multidimensional scaling routine (INDSCALE) which will group the stutterers on the basis of the speech pathologists' responses. The scaling data are then compared to the measurements of the stuttered speech to determine which dimensions form similar groupings.

Progress: Twenty-five stutterers have been recorded, but only eight of these have been selected as subjects. The remaining seventeen stutterers did not stutter or stuttered very little during the reading task and thus were eliminated. The following measurements were made from the recordings of the eight subjects: instances of stuttering, instances of sound repetitions, instances of syllable repetitions, instances of word repetitions, interjections, number of sound repetitions, number of syllable repetitions, number of word repetitions, number of phrase repetitions, duration of prolongations, duration of pauses, total reading time, fundamental vocal frequency, and intensity. It is anticipated that the two remaining stutterers need for the project will be recorded in the near future.

The rationale for this project is that judgments of stuttering severity are based on several dimensions of the speech behavior. The data to date give face validity to this rationale in that the subjects show a great deal of variation in most of the parameters measured. In addition, the data reveal that subjects who are low on one parameter are high on other parameters. These results indicate that although a stutterer may be mild on one dimension of speech, he may be a severe stutterer if another dimension is used as a measure. Fundamental vocal frequency and intensity showed very little variation either within or between subjects. As a result, these measures have been eliminated from the study.

Conclusions: Not applicable at the present time.

Funds Utilized, FY'76: \$3540.00 B&K Graphic Level Recorder  
\$216.00 Supplies

Funding Requirements, FY'77: None

Type of Report: Interim

Work Unit No.: 2511

Title: Memory for Consonants of Normal and Hearing-Impaired Observers

Investigators:

Principal: Brian E. Walden, CPT, MSC

Associate: Robert A. Prosek, CPT, MSC  
Allen A. Montgomery, Ph.D.

Objective: To determine the influence of peripheral hearing loss on the coding of speech stimuli in memory.

Technical Approach: Pairs of consonant-vowel stimuli are presented to subjects for similarity judgments. Five of the subjects have normal hearing, five have moderate-to-severe adventitious hearing losses, and five have severe congenital hearing impairments. Using an equally appearing interval scale, the subjects rate the similarity of the consonant pairs presented auditorily, and visually via an orthographic display. The resulting data are analyzed to reveal differences between the groups and between the modes of presentation.

Progress and Results: Thus far, data have been obtained from five normal-hearing subjects and five subjects with congenital impairments. Only preliminary statistical analyses have been completed. These analyses suggest that the ratings of the normal-hearing subjects are quite comparable for both modes of presentation. Further, they demonstrate acceptable test-retest reliability, as do the subjects with congenital impairments.

Conclusions: N/A

Funds Utilized FY-76:	\$138.00	- Subject pay
	<u>\$325.85</u>	- TV monitor
	<u>\$463.85</u>	- Total

Funding Requested FY-77: None

Publications: N/A

Type of Report: Interim

Work Unit No: 2512

Title: The Effects of Hearing Impairment and Acoustic Filtering  
on the Perception of Speech

Investigators:

Principal: Brian E. Walden, CPT, MSC

Associate: Robert A. Prosek, CPT, MSC  
Allen A. Montgomery, Ph.D.

Objective: The objective of this experiment is to describe those effects of hearing loss on speech perception which cannot be accounted for on the basis of the frequency distortion imposed by reduced auditory sensitivity. Specifically, the purpose is to determine which speech sounds (or classes of sounds) are perceived similarly and differently through an impaired ear and a normal ear listening through a filter network which has been matched to the impaired ear's audiometric configuration.

Technical Approach: Consonant confusion matrices will be constructed for 20 adults with unilateral hearing impairments. For the impaired ear, the consonants will be presented without any external distortion. For the normal ear, however, the stimuli will be presented through a multifilter network which is adjusted to match the configuration of the hearing loss in the impaired ear. In addition, pairs of consonants will be presented sequentially to the two ears for judgments of consonant similarity using a equally-appearing interval scale. The resulting data will be analyzed to reveal which consonants (or classes of consonants) are perceived differently through the impaired ear and filter network.

Progress and Results: Both the 400-item confusion matrix test tape and the 80-item paired-comparison tape recording have been prepared. All equipment has been received except for the Spectrum Shaper and the Combining Unit. These are scheduled for delivery on or about 19 July 1976. Once this instrumentation is received, data gathering can begin.

Conclusions: N/A

Funds Utilized FY-76:	\$4157.80	- Graphic level recorder
	\$3117.00	- Beat-frequency oscillator
	<u>\$6161.80</u>	- Spectrometer
	\$13436.60	- Total*

\*Total does not include cost of equipment not yet received.

Funds Requested FY-77: None

Publications: N/A

Type of Report: Interim

WORK UNIT NO. 2601

TITLE: In vitro and in vivo properties of sensitized lymphocytes

INVESTIGATORS:

Principal: E.K. Spees, M.D., COL, MC

Associates: C.R. Annable, M.D., COL, MC  
C.R. Reckard, M.D., LTC, MC  
D. Oakes, M.D., MAJ, MC  
C. Alving, M.D., LTC, MC

OBJECTIVES: To define the kinetics and mechanisms of lymphocyte immune responsiveness to antigens, including allogeneic cells and a variety of other antigens in vitro and in vivo.

TECHNICAL APPROACH: This study uses blood components to predict and quantitate the human immune response to tissue or organ allografts. These assays are of particular importance in subjects with end stage renal disease who will receive a renal allograft, subjects with diabetes mellitus who will receive islet cell transplants, persons with neoplastic diseases scheduled for radio-, chemo-, or surgical therapy, and subjects with various immunodeficiency disorders requiring bone marrow transplantation or other treatment. A variety of different assay techniques have been adopted to quantitate cell mediated immunity (CMI) as well as humoral immunity in these subjects.

a. Immunogenetic studies include serological HLA tissue typing, mixed lymphocyte culture studies, and cell mediated lympholysis. The tests allow a comparison of "donor" and "recipient" cellular compatibility, and may assist in the selection of a living or cadaver organ or tissue donor with the best chance of survival in the recipient. The leukocyte crossmatch is a valuable ancillary test in preventing transplantation in the face of preformed recipient antibodies against the donor cells.

The ability of a subject to respond to a standard battery of skin antigens is often impaired or lost in patients with end stage renal disease, malignant disorders and various types of immunodeficiency states. We have been recording in vitro lymphocyte blastogenesis and migration inhibition factor (MIF) after antigen stimulation to quantitate the ability of subjects to respond to antigens by delayed hypersensitivity. A new method for testing responsiveness to 2,4-dinitrochlorobenzene (DNCB) has been developed which may obviate the need to apply skin tests, since the assay can be carried out completely in vitro and corresponds nicely to in vivo results. This assay utilized in vitro sensitization to DNCB-labelled carriers including phospholipid microspheres, autologous leukocytes, platelets, or membrane stroma material.

A successful method of intraportal pancreatic islet cell transplantation has been developed in which islet cell clumps are separated by collagenase digestion from whole pancreas and enrich to high purity prior to injection. Allograft rejection response can be easily measured in the diabetic islet cell recipient

rodents, since rejection of the endocrine graft causes a rapid and permanent elevation of the blood glucose level. Allograft acceptance, on the other hand, allows maintenance of nondiabetic serum glucose levels.

The importance of the traffic pattern of splenic lymphocytes on allograft rejection and graft - versus host (GVH) disease has only recently been appreciated. Assays to quantitate this matter in rodents include the pretreatment of rat spleen donors with skin grafts or leukocyte antigen preparations isogeneic to the graft recipient strain.

If the donor spleens are removed a short time later, the spleen cells show reduced cell mediated immunity potential against the recipient in a GVH assay, since the lymphocyte subpopulations acting against the recipient are away from the spleen at the time of splenectomy.

All of the aforementioned diagnostic assays are conducted at WRAMC or WRAIR except the MIF assays, which are done by Dr. James Ballow at NIH. No experimental drugs are used. All human subjects execute a volunteer agreement with overprint in accordance with WRAMC 70-1.

#### PROJECTS AND RESULTS:

<u>Active Subprojects</u>	<u>Code</u>
A. Discriminative MLC	DMLC
B. Lymphocyte mediated cytotoxicity	LMC
C. Multilayer hypaque-ficoll gradient	MHFG
D. Phospholipid microsphere carriers	PMC
E. Isolation and transplantation of isolated pancreatic islets	ITIPI
F. Splenic subpopulations-GVH	SS-GVH
G. <u>Ex vivo</u> organ perfusion immunity	EVOP

#### Inactive Subprojects

H. Leukocyte aggregation test	LAT
I. Radioinactivation of stimulator cells	RISC

A. Discriminative MLC (DMLC): This assay is used in all potential living related donor renal transplants. In the case of serologically "HLA identical" transplant pairs, the method can detect a genetic recombination on the 6th chromosome between the HLA antigen and MLR locus. This is valuable because it predicts that allograft rejection may occur, and that prognosis of the graft is poorer than the usual HLA identical case. During FY 76 none of our 3 HLA identical graft recipients showed MLC stimulation to their donors lymphocytes, and all grafts have functioned well. One patient had an early reversible rejection. DMLC is also useful in detecting antibody-mediated blocking of recipient responses against donor cells, a phenomenon often seen in longterm surviving allograft recipients. None of our recipients have shown this phenomenon during FY 76. DMLC, HLA tissue typing, and leukocyte crossmatching will remain our basic screening tests in selection of histocompatible donor-recipient pairs for some time to come.

B. Lymphocyte mediated cytotoxicity (LMC): This assay appears to detect the ability of effector lymphocytes either preformed in vivo in an individual or generated by in vitro culture with allogeneic cells to specifically kill allogeneic lymphocytes that have been labelled with  $^{51}\text{Cr}$  radioisotope. Eight kidney recipient candidates were studied with this assay during FY 76. Three HLA identical pairs showed no cytotoxic potential against their donors. Five other recipient candidates were tested and four have been transplanted. All four recipients had absent cytotoxic potential against the donors, but present against random allogeneic donors. Two renal recipients have had good allograft acceptance, but the other two had allograft rejections requiring removal of the kidney.

Recent publications have suggested that the LMC assay may be one of the best prognostic tests, since the absence of cytotoxic activity against donor lymphocytes but not other allogeneic cells has been found to be the common denominator in successful long term renal transplant recipients. We have found this to be invariably true in all the HLA identical subjects we have tested. We intend to retest our transplanted patients annually to confirm this fact.

C. Multiple hypaque-ficoll gradient (MHFG): This technique has been used on numerous occasions to separate subpopulations of lymphocytes from peripheral blood by their physical sedimentation velocity features. The resultant layers of cells can then be tested for their proportions of B and T lymphocytes by immunofluorescent or mitogenic responses in vitro. This has been useful in determining which lymphocyte subpopulations are responsible for the phenomena seen in DMLC, LMC, and antigen stimulation assays.

D. Phospholipid microsphere carriers (PMC): Significant progress was made with PMC studies during FY 76. Dr. C. Alving at WRAIR prepared many different varieties of PMC's for in vitro assays. Our most interesting result was the discovery that we could induce in vitro primary sensitization of leukocytes to DNCB-labeled PMC's, and show lymphocyte blastogenic kinetics parallel to those we have shown after skin application of DNCB. Since delayed hypersensitivity to DNCB skin application is known to be a bad prognostic factor in renal transplant recipients and a good sign in patients with cancer, and immunodeficiency, we are restudying many of our renal transplant recipients to determine the reliability of this phenomenon. We have also used the assay to follow head and neck cancer patients who are skin tested by Dr. C. Casterline of the Allergy Clinic as a regular part of their evaluation.

In vitro responsiveness to PPD, candida, SKSD, and other antigens has been standard for years, but in vitro DNCB testing has been impractical due to the toxic properties of the chemical. Attaching the DNCB to PMC's makes DNCB nontoxic according to our observations, although we have observed some nonspecific mitogenic effects if too high a concentration of DNCB-labelled PMC's is used. After in vitro sensitization to the PMC's the specific blastogenesis peaks at about ten days and then gradually subsides to low levels, just as we see occurring after cutaneous application of DNCB.

The principal investigator is hoping to encounter subjects who do not respond to DNCB, an effect frequently reported in end stage renal disease subjects and cancer patients. If such subjects are found, *in vitro* investigations of the defect in cell-mediated immunity are planned, including the utilization of RNA extracted from DNCB-immunized guinea pig tissues with the assistance of Dr. Y. Pilch of Harbor General Hospital, Torrance, CA. If the immunodeficiency of the subjects is a blockage of specific RNA production, then there is reason to believe that the exogenous RNA may be able to convert the cells from nonresponder to responder status in culture. This would be an important breakthrough in the field.

E. Isolation and transplantation of isolated pancreatic islets (ITIPI): Since the progress report submitted in April, 1975, our laboratory has further defined the immunologic advantages of the intraportal implantation site. Using AgB incompatible inbred rat strains, our current data suggest that using the same immunosuppressive protocol, intraportal islets enjoy much more prolonged survival than do islets transplanted intraperitoneally. At the current time, approximately 50 transplants have been done in this aspect of the project. The immunosuppressive agent has been horse anti-rat lymphocyte serum. We are currently in the process of making more anti-lymphocyte serum, so that greater numbers of transplants can be performed to make the differences more statistically significant. Moreover, it is planned that skin grafts will be performed in this same donor-recipient combination to measure and compare their survival to that of islets, using the same immunosuppressive protocol.

Using the protocol suggested by Dr. Frank Stuart, our laboratory has made recipient anti-donor antibody (Lewis anti-BN antiserum). This has been tested in Dr. Stuart's laboratory at the University of Chicago, and been found to have significant enhancing activity in his kidney transplant model. Following his protocol, which has been shown to markedly prolong kidney allograft survival, to date we have been unable to enhance the survival of isolated islet allografts. This has been performed in approximately 12 animals without success, suggesting that the islets, which are cell aggregates, are not as readily enhances as are whole organ grafts (kidneys). During the next several months, we intend to pursue this project at the laboratory here, in collaboration with the University of Chicago.

At the current time we are investigating the effect which the site of implantation has upon the responsiveness of islets to GI hormones, namely secretin. It has been reported by Weber et al at Columbia, that intraperitoneal islet recipients do not respond to IV secretin with an increase in insulin output, as do normal animals. They have suggested that the reason for this is that islets require an intact exocrine pancreatic milieu. *In vitro* work on this point has been conflicting. We have postulated that it is not necessary for islets to have an intact exocrine envelope for a normal response to secretin. However, it may be that the islets must remain within the portal outflow tract. Therefore we are currently measuring the insulin response to secretin in normal rats, recipients or either intraperitoneal or intraportal islet isografts, and normal animals. These values will be compared to either substantiate or disprove our hypothesis. Insulin levels are being measured in Dr. Arthur Rubenstein's laboratory at the University of Chicago and are not available as of this date.

F. Splenic subpopulations-GVH (SS-GVH): Effects of splenectomy on transplantation immunity and immunological enhancement - although the effects of splenectomy on antibody responses have been firmly established, no conclusive evidence of changes in cellular immune responses have been demonstrated, and there are some conflicting opinions on the role the spleen in immunological enhancement. Induction of enhancement to tumor grafts has long been known to be suppressed following splenectomy and more recently it has been reported that neither active nor passive enhancement can be achieved in splenectomized animals.

Splenectomy has been carried out preceding clinical kidney transplantation on a basis of the general concept that removal of the spleen will diminish the immune capacity and/or shift the balance between humoral and cellular immune responses of tissues; and, to remove the inhibitory influence that the spleen has on bone marrow production of the cellular components of blood and therefore allow for a greater use of immunosuppressive drugs.

Using a standard skin grafting model in the rat, experiments over the past two years indicate that splenectomy will prolong graft survival and that contrary to recent conclusions, active enhancement can be achieved, however, only by intravenous immunization procedures. Enhancement antibody responses were achieved, however, only by increasing the antigen load 30 fold. The time sequence of antibody responses in the splenectomized animal were not different from that in an intact animal, and the best enhancing antibody response was present at 10 to 14 days following the last antigen injection. In addition to the suppressive effects of pre-grafting procedures, splenectomy prior to the onset of cellular rejection reactions of histoincompatible grafted tissues also markedly delays the immunologically mediated rejection of allografts. In other words, splenectomy 48 to 96 hours following transplantation more than doubles the survival time of the graft. The curious result that splenectomy 24 hours following an immunization procedure does not alter the immune response of the rat is made clear by the results of studies we have done this year on lymphocyte kinetics and which are explained more fully in the next section. Briefly, these results demonstrate that the specific antigen sensitive lymphocyte population is mobilized from the spleen by 24 hours and returns to that organ in increasing numbers over the following three to four days. (Our limited knowledge of the effects of splenectomy in humans when compared with these animal studies indicates that the antibody response to intravenously administered antigens is the same and that for infants and children, but not adults, cellular immune responses probably also parallel the responses found in the rat model).

Obviating the Graft Versus Host Reaction - The severity of the graft-versus-host reaction is usually heightened and its onset accelerated if the offending cellular inoculum is obtained from specifically presensitized donors. However, in studies of lymphocyte kinetics during the early induction phases of an immune response, experiments completed this year now show that donor bone marrow, or lymph node, and splenic lymphoid cells can be rendered immunologically unreactive in a standard graft-versus-host reaction model following the intravenous injection of cellular antigens that are isogenic to the prospective host and allogenic to the donor. This reaction has been found to be antigen specific and the lymphoid cellular preparations give a full graft-versus-host response when antigens not isogenic to the intended host and also allogeneic to the donor are injected prior to the

harvesting of the bone marrow lymphoid organs. The time sequence of these events is that by 24 hours the specific antigen reactive lymphocytes have been fully mobilized, by an adequate antigen load, from the "home" lymphoid tissues and from the recirculating pool of lymphocytes. Between 48 and 96 hours following the antigenic exposure, there is a gradual return of specifically reactive cells to the bone marrow, lymph nodes, spleen and recirculating lymphocyte pool. By 96 to 120 hours some hyper-reactivity is present; such as would be expected in the pre-sensitized donor. This model demonstrates a potential method for performing bone marrow transplantation using a histoincompatible donor while obviating the lethal graft-versus-host reaction that is the normal outcome of such a grafting procedure. This proposition for successful marrow transplantation across major histocompatibility barriers will be tested in the coming year(s).

G. Ex vivo organ perfusion immunity (EVOP): Clinical studies - The principle successful clinical support over this past year has been the preparation of rabbit anti-horse and anti-goat sera for a monitoring the course of horse and goat anti-human lymphocyte globulin immunosuppressive therapy in patients on the organ transplantation service and carrying out the assays for the presence of horse (or goat) globulins in the patients' serum and for testing for patient anti-horse antibodies.

Immunofluorescent and antibody elution studies on a couple of transplanted kidneys that had unusual early rejections characterized by interstitial hemorrhage and parenchymal necrosis did not lead to any significant insights as to the cause for the organ transplantation failures.

Florescein conjugation of specific antibody preparations for direct immunofluorescent studies was done as a technical service for other laboratories at WRAMC and WRAIR.

H. Leukocyte aggregation test (LAT): This assay has been inactive during FY 76 because of the absence of our technician Mr. Felix Passaretti on Workmen's Compensation for the entire period. Reactivation of the project is anticipated upon his return.

I. Radioinactivation of stimulator cells (RISC): This project has been inactive during FY 76 due to its lower interest priority in comparison to the projects described above. Work may be resumed on it if time permits.

**CONCLUSIONS:** In vitro assays of lymphocyte responsiveness to allogeneic cells and various antigens used in this research project have provided valuable clinical donor selection and allograft prognostic criteria in renal transplantation patients. An especially promising new assay using DNCB-labelled microspheres has been developed in our laboratory and appears to be of great potential value in measuring delayed hypersensitivity responsiveness in patients with end stage renal disease, cancer, and immunodeficiency diseases.

New insights have been developed regarding the immigration of immunocompetent spleen cells. This information could possibly be used to devise a strategy for more successful bone marrow transplantation when a histoincompatible bone marrow donor is utilized.

The rodent pancreatic islet cell transplantation work from our laboratory is necessary ground work for the eventual widespread use of this method in human diabetic subjects. Before human use is possible, we must solve the problem of how to cultivate pancreatic cells in large numbers, and how to cryopreserve the cells for long term storage until needed.

We have made our lymphocyte responsiveness assays freely available to other clinical services for assistance in day to day clinical patient care.

FUNDS UTILIZED, FY76: \$66,634.02

FUNDING REQUIREMENTS: Previously submitted.

PUBLICATIONS: There are several publications in progress but none have been published.

TYPE OF REPORT: Interim.

WORK UNIT NO. 2610

Title of Project: Antilymphocyte Globulin and Kidney Transplantation: A Controlled Double Blind Study

Investigator: Jimmy A. Light, LTC, MC

Objectives: To define the affects of ALG on the rejection of kidney transplants from live donors to recipients with quantitated *in vitro* histo-incompatibility; To examine effects of ALG cellular immune defense mechanisms and on the coagulation system.

Technical Approach: Double blind administration of ALG or placebo to recipients selected prospectively.

Progress: This study has not yet commenced because of the difficulty in locating a sufficient quantity of high quality antilymphocyte globulin that is free of major sideaffects and of uniform effectiveness.

Conclusions: The study is still worthwhile and the project design is good. We recommend continuation of the project into FY 77 and in an active status. Meanwhile further efforts to resolve the ALG supply situation will be continued.

Funding Requirements: None.

WORK UNIT NO: 2611

TITLE OF PROJECT: Survey of Multiparous Patients for Anti HL-A Antibodies

INVESTIGATORS: Everett K. Spees, Jr., COL, MC

Associate: None

OBJECTIVES: To survey multiparous females for HLA antibodies suitable for reagent use.

TECHNICAL APPROACH:

PROGRESS & RESULTS: No work was completed on this project due to lack of personnel.

CONCLUSIONS: N/A

FUNDS UTILIZED, FY-76: None

FUNDING REQUIREMENTS, FY 77:

Personnel: None

Equipment: None

Supplies: \$600.00

Travel: None

Other: None

PUBLICATIONS: None

TYPE OF REPORT: Interim

WORK UNIT NO: 2612

TITLE: Routine Use of Antilymphocyte Globulin in Recipients of Renal Allograft

INVESTIGATOR: Jimmy A. Light, LTC, MC

PROGRESS AND RESULTS: The request for approval to use an investigational drug Antilymphocyte Globulin in kidney transplant recipients on a routine clinical basis was first submitted in summer 1975. The request was never finally approved. There was considerable confusion over language and confusion with a previously approved project on ALG and kidney transplantation, Work Unit No. 2610. In addition, the AIDRB had requested certain information from the primary investigator, the University of Minnesota, which they have never submitted despite repeated requests.

While awaiting final approval twelve patients were treated with the Antilymphocyte Globulin material, with individual approval being obtained for each one. Final summaries on each of these patients were forwarded in June 1976, and are attached. An appraisal of the treatment results in these 12 patients fails to demonstrate any dramatic change in the success rate of kidney transplantation at Walter Reed Army Medical Center.

In view of the difficulty obtaining necessary information from the primary investigator, and in view of the lack of a clear cut beneficial affect of the agent on patients receiving kidney transplantation at WRAMC, I recommend that Work Unit No. 2612 be discontinued. I recommend that in future situations where Antilymphocyte Globulin therapy may be indicated that modifications to the existing approved protocol Work Unit No. 2610 be used instead of new applications for research projects.

WORK UNIT NO. 2613

TITLE: Long Term Pulmonary Function Following Recovery from Pneumocystis Carinii Pneumonia

INVESTIGATOR: David D. Oakes, Major, MC

PROGRESS & RESULTS: The application for approval of this project was considered by the Human Use Review Office, Office of The Surgeon General. Their comments are contained in the attached Disposition Form dtd 27 Aug 76.

The implementation of this study has been delayed pending approval of the Human Use Review Office and the arrival of new, specialized equipment in the pulmonary function laboratory. Now that approval has been obtained and the equipment installed, the study should proceed within the next month.

Work Unit No.: 2700

Title of Project

Thoracic Surgery Retrospective and Prospective Clinical Research Projects

Investigators

Principal: COL David C. Green, MC

Associate: COL Walter H. Brott, MC  
LTC Thomas E. Bowen, MC  
LTC John A. Eielson, MC  
LTC Roy L. Kingry, MC  
MAJ Ross S. Davies, MC

Objectives

(1) To measure parameters of physiologic change occurring in the dynamic course of patients undergoing pulmonary and cardiac procedures so as to evaluate technical advances applied to their care. (2) To continue an ongoing collection of data in the large series of patients treated or under treatment by the Thoracic and Cardiovascular Service. (3) Correlation and extraction of follow-up data with eventual computerization of information in order to derive valid conclusions.

Technical Approach

Separate research files containing pertinent preoperative, operative and postoperative data are kept on all cardiovascular and many pulmonary surgical patients. The files now include over 3000 patients. Extraction of this data in various patient disease categories is being performed by thoracic surgeons with the help of the research secretary. Continuing long-term follow-up is being established. Input of data into computers and development of mechanisms for better long-term follow-up data collections are anticipated. Coordination with the computer systems for the new hospital has gone into the first phases with the computer technologists. This is a continuous, ongoing project for the future.

Progress & Results

Continued progress has been accomplished in organization, updating information and collection of new data on current and old patients (dating back to 1958). Approximately 350 new cases have been added and additional material added to over 1500 files. Continued efforts to make follow-up as close to 100% as possible was made by newer follow-up administrative procedures. Two groups of patients (those with the Kay-Shiley mitral valve and those with the Starr-Edwards 1000 and 2320 valves) have been brought to that 100% level with analysis of these series having great import. Replacement of the ball poppet or the whole valve in the Starr 1000 series valve has been accomplished in our appropriate patients. The patients with the 2320 valve have had a change in their antithrombosis prophylactic regimen based on the analysis of their follow-up data. This represents

pioneer information gathered and will be reported at the annual Army Cardiology meeting by Dr. Brott, Dr. Green and Dr. Bowen. The Kay-Shiley mitral valve study is also of significance as the largest series of this type of valve with 100% follow-up.

In order to accomplish the follow-up of multiple groups of patients (including aortic valve replacement, mitral valve replacement, coronary artery bypass surgery, coronary artery anomalies, tracheal injuries, traumatic aortic aneurysms, traumatic bronchopleural fistula, carcinoma of the esophagus, VSD and aortic insufficiency, postoperative atelectasis, pulmonary sequestration, cardiac pacemakers, tetralogy of Fallot, aortic arch anomalies, pulmonary function related to surgery, pseudotruncus, double outlet right ventricle, azygous continuation of the inferior vena cava and, most recently, use of autologous blood for replacement in open heart surgery), hundreds of letters and telephone calls are required to complete follow-up. As data becomes complete enough for analysis, this is performed. Data collected has been used directly for formulating therapy for whole groups of patients such as with the SE 1000 and 2320 valves. This information is also passed on to other physicians through medical channels.

#### Conclusions

Multiple projects related to direct patient follow-up subsequent to surgical procedures of general thoracic and cardiac nature have been initiated and ongoing. Results from several of the projects have been presented at military and civilian medical meetings, and some are published or in the process of being published. These have significantly enhanced medical and surgical knowledge in the Army Thoracic Program as well as in the thoracic specialty in general. A large volume of clinical data remains uncataloged and ripe for clinical research studies. Proper and meaningful follow-up with extension to those patients who do not have clinical follow-up at Walter Reed and therefore can have their data followed only by mail or other contacts requires a continuing effort once initiated. These projects cannot be continued without the full-time support of a research secretary. The eventual goal will be the transfer of the data from our over 3000 research files to computers with updating of follow-up for rapidly attainable, meaningful clinical research data on all the cardiac diseases. In a dynamic field, frequent evaluation of results and new methods is imperative in any progress such as Walter Reed's which generates statistically valid numbers of cases.

<u>Funding Requirements</u>	<u>FY 1976</u>	<u>FY 1977</u>
Personnel: Alveria Cole	\$9,521	
Equipment:	1,400	
Supplies:		\$300
Travel:		
Other:		
Printing and reproductions:	200	200

Work Unit No.: 2701

Title of Project

Comparison of the Effect on Cardiac Muscle Physiology by Protection with Coronary Perfusion as Opposed to Local Hypothermia during Cross-clamping of the Aorta

Investigators

Principal: COL Walter H. Brott, MC

Associates: COL David C. Green, MC  
CPT Joseph P. Mandl, MSC

Objectives

To compare parameters of cardiac muscle physiology (particularly evidence of anaerobic metabolism) while using the two most common modes of protection of the myocardium during cross-clamping of the aorta while on cardiopulmonary bypass.

Technical Approach

While on cardiopulmonary bypass using systemic hypothermia with either topical profound myocardial cooling or coronary artery perfusion, samples every 10 minutes are taken from coronary sinus blood for lactate, pyruvate, CPK, PCO<sub>2</sub> and PO<sub>2</sub>. Simultaneous tissue PO<sub>2</sub> and PCO<sub>2</sub> are measured with a mass spectrometer tissue probe in the myocardium with temperature measure for temperature correction.

Progress and Results

Eight patients have had satisfactory studies performed from the onset of the project. Problems related to calibration of gas tensions to low temperatures have negated some of the other patient results. With no further support by the Ft. Dietrick lab for the CPK and lactate determinations, this portion of the study came to a standstill. Maintenance support was not present for the mass spectrometer; therefore, calibrated studies could not be done. One study was made with a loaned PO<sub>2</sub> probe in one patient in an effort to convert to a more maintenance-free system. Further study of this and requests related to this thermocouple probe will be made to reduce maintenance costs and make the project more workable.

Conclusions

(a) The trend of recorded results so far has verified myocardial protection by profound topical hypothermia. As rewarming as initiated one first sees significant products of anaerobic metabolism as well as elevation of CPK, indicating ischemia. (b) In one case the studies made the diagnosis of partial occlusion of the left coronary artery by a prosthesis as the normal return after release of the cross-clamp did not ensue. The aorta was reopened and the valve reseated. (c) Approximately seven more studies are

required to complete the project. The applicability of doing the study depends on appropriate anatomy and muscle thickness. Previous surgery with adhesions makes placement of the electrodes undesirable if the coronary branches cannot be seen to be avoided. Conversion to a thermo-couple probe (IBC VIVOX PO Electrode) so as not to require the mass spectrometer is presently under consideration. To do this while making the previous results comparable and valid with new data, comparison use of both in at least three patients is required. Therefore, a contract for maintenance on the mass spectrometer will be required this year.

Funding Requirements

FY 1976    FY 1977

Other: Maintenance contract - CMBLOG 2262  
103-012-1400

\$1,500    \$1,500

Publications or Papers: None

Work Unit No.: 2702

Title: Postoperative Open Heart in Vivo Measurements of Partial Pressures of Oxygen and Carbon Dioxide by Mass Spectrometry.

Principal Investigator: LTC A. W. Fleming, MC  
Dept of Surgery  
Thoracic Surgery Svc

Status: Several requests for the Annual Progress Report on this project have been ignored. The Clinical Investigation Committee in a meeting on 29 September 1976, terminated this study.

Work Unit No: 2703

Title of Project: Exclusive use of autologous blood transfusions (autotransfusions) in elective thoracic and open heart surgical procedures.

Investigators:

Principal: Arthur W. Fleming, MD, LTC, MC  
Associates: Daria St. James, CPT, ANC  
David C. Green, MD, COL, MC  
John H. Radcliffe, MAJ, MSC  
Peter Olson, MD, MAJ, MC

Objective: To develop a systematic approach for obtaining a sufficient volume of autologous blood for use during and after all elective thoracic and open heart surgical procedures, thus eliminating the need for homologous blood transfusions.

Technical Approach: Each unit of blood was collected in CPD preservative by AABB standards. Blood collected from donors for open heart surgery was separated into packed cells and plasma. The packed cells were then frozen if the period between donation and surgery was greater than 3 weeks; or stored at 4°C if the interval between donation and surgery was less than 3 weeks. The plasma was frozen regardless of the time interval for open heart surgical procedures. The blood from donors not requiring extracorporeal circulation was maintained as whole blood if the interval between donation and surgery was less than 3 weeks. If the interval between donation and surgery was greater than 3 weeks, the blood was treated in the same manner as for open heart surgery, i.e., the unit of blood was separated into packed cells and plasma, and then both components were frozen. Frozen packed cells were thawed on the afternoon prior to surgery, and the plasma was thawed on the day of surgery. One to two additional units were drawn intraoperatively on open heart surgical cases and the units maintained at room temperature until the completion of cardiopulmonary bypass. These units were then transfused back to the patient in the immediate post-pump period.

Progress and Results: Up to 10 units of blood have been obtained safely using a combination of preoperative and intraoperative phlebotomies. Many of the open heart surgery patients donated 50 to 90% of the total blood used during their hospitalization. None of the patients who have been asked to donate blood for their operation have refused. No serious complications have occurred in patients who have predeposited blood for their own operation. Further progress has been made in the design and development of forms so that information can be disseminated to the anesthesiologist, operating room technicians, recovery room nurse, ward nurses and doctors in an expeditious manner.

Conclusions: Utilizing the techniques outlined above, we were able to accomplish the following: The risk of creating a significant hypovolemia was virtually eliminated by extending the interval between phlebotomies; the total volume of homologous blood transfused was reduced by the use of autologous fresh frozen plasma, fresh whole blood and fresh frozen red blood cells; the incidence of hepatitis was reduced; some of the logistical problems in obtaining certain blood types were diminished; and the drain on blood bank stores was decreased.

Funds Utilized FY-76: An exhibit bearing the same title was presented to the American Association of Blood Banks in November, 1975 (TDY-5 days).

Funding Requested FY-77: Travel: \$500.00.

Publications (FY-76): None.

Type of Report: Interim

Work Unit No.: 2804

Title: An Evaluation of the Efficacy of Tadenan in the Treatment of Benign Prostatic Hyperplasia.

Principal Investigator: Bernhard T. Mittemeyer, COL MC  
Chief, Urology Service

Objective: To determine whether or not Tadenan would be effective in relieving symptoms of benign prostatic hyperplasia as an alternative to surgery.

Technical Approach: The study has as yet not been initiated.

Progress & Results: The study has not yet been initiated.

Conclusions: The study has as yet not been initiated.

Funds Utilized FY-76: The study has not been initiated yet.

Funds Requested FY-77: The study has not been initiated.

Publications: The study has as yet not been initiated.

Type of Report: The study has as yet not been initiated.

Additional studies are being done by Bionetics Laboratory in Bethesda to satisfy FDA requirements prior to human use. Upon completion of these studies, we will be advised and appropriate clinical trials will be instituted.

Work Unit No.: 2805

Title of Project: Biochemical Studies of Urinary Polyamines  
in Human Genitourinary Carcinoma.

Principal Investigators: H. David Cox, MD, MAJ MC; B. P.  
Doctor, PhD; B. T. Mittemeyer, MD  
COL MC

Co-Investigators: David McLeod, MD LTC MC; William  
McDonald, MD MAJ MC, William Belville, MD  
MAJ MC

Objective: To determine new methodology in the study of  
urinary polyamines in urine and serum.

Technical Approach: Urine is taken from patients having  
genitourinary carcinoma prior to any definitive therapy.  
The patients are then treated in a routine fashion for their  
particular disease and at specific points in time follow up  
urines are obtained. The purpose of the investigation is to  
specifically identify increases or decreases of urinary  
polyamines and compare these changes to changes in their  
clinical course, i.e., remission and/or exacerbation of  
disease.

Progress and Results: Grant effort was extended in an  
attempt to extract urinary polyamines and derivitize them so  
that they could be analyzed by gas liquid chromatography.  
While some advances have been made, the published  
methodology of the derivitization has not proved  
satisfactory for the rapid analysis of urinary polyamines.  
Backup methodology of an amino acid analyzer was employed  
because of the disappointing results with gas liquid  
chromatography. Utilizing a JEOL 6 AH amino acid analyzer,  
we have developed new methodology for the measurement of  
urinary polyamines. This has included a three buffer system  
with a modified ninhydrin reagent and DMSO as a solvent  
system. We have developed a program which allows continuous  
applications of samples to the amino acid analyzer without  
stopping. We have identified other diamines in human  
urines, e.g., 1, 3 diamino propane which is breakdown  
product of spermidine. We have also resolved and identified  
cadavarene which has heretofore not been described in the  
urine of patients with carcinoma.

Work Unit #2805 (continued)

**Conclusions:** We have developed a new methodology of extraction and measurement of urinary polyamines by an amino analyzer which allows lower operating pressures, reduced buffer flow and ease of operation. We have identified for the first time, two compounds in the urine of patients with carcinoma of the genitourinary system. Both of these compounds in addition to spermine, speridine and sultescine have been found to be elevated in our measurements of some patients with genitourinary carcinoma. We have every reason to continue to believe that these compounds may be utilized as tumor markers in the diagnosis, staging and prognosis of patients with genitourinary carcinoma.

**Funds Utilized:** FY-76: Less than \$2,000, Funding requested for FY-77: Equipment and supplies, \$1,500.

**Publications:** We are presently submitting a publication to the Journal of Chromatography on the new methodology that we have developed.

**Type of Report:** Interim

Work Unit No.: 2901

Title of Project: The Use of Proplast in Soft Tissue and Bony Augmentation

Investigator:

Principal: Douglas S. Rowe, MAJ, MC

Date of Approval (OTSG): 25 Sept 74

Date of Approval (WRAMC): 15 Aug 74

Objectives: We plan to establish the utility in place of Proplast as a new implant material. We are investigating its use because of a reported ease of sculpturing and rapid stablization by surrounding tissue. It is not our intent to draw statistical correlations with other implant materials.

Technical Approach: We are currently using this alloplastic material making special note of its handling characteristics at the time of operation, following the patients clinically along with x-ray and laboratory studies as outlined in the protocol for this project.

Progress & Results: We presently have 5 patients on the protocol. All of the cases have been bony augmentations of the facial skeleton. To date there have been no problems with the implant material. There have been no infections or extrusions. Since this protocol was initiated Proplast has been brought on the market for a general use and it is no longer considered an investigational material.

Conclusion: To date the Proplast has fulfilled its expectations as an excellent implant material. It is easily carved, rapidly fixed, and biologically inert.

Funding Requirements: No special funding will be required. The continued support of Radiology and the Laboratory will be necessary as outlined in the original protocol.

Publications: During the past fiscal year no data has been submitted for publication.

Type of Report: Termination

Work Unit No.: 3102

Title of Project: Therapy of Immunodeficiency Diseases with Transfer Factor

Investigators:

Principal: Arnold Levinson, M.D. MAJ MC

Associate: Richard Evans, III, M.D. LTC MC

Objective: Transfer factor, a low molecular weight substance extracted from leukocytes, has been used by us and others to reconstitute T-cell function in patients with a variety of primary and secondary immune deficiencies. These patients are identified by exhaustive immunologic evaluation as described in Protocol 3317 and therapeutic intervention undertaken when evidence of T-cell deficiency is demonstrated.

Technical Approach: Transfer factor has been prepared as outlined in our original protocol with the aid of the hematology group.

Progress and Results: Mark Ballow, M.D. MAJ MC has departed from Walter Reed Army Medical Center and the new principal is Arnold Levinson, M.D. MAJ MC. Since our last interim report, we have continued to intensively treat three patients with transfer factor. The first, an eight year old girl with chronic mucocutaneous candidiasis, has continued on high dose transfer factor in addition to thymus transplantation (latter performed under Protocol 3103). This patient continued to show abnormal in vitro responsiveness to specific antigens despite high dose transfer factor therapy. In addition, clinical remission became progressively shorter following clearance of skin lesions with amphotericin B therapy. Not until combination transfer factor therapy and thymus transplantation were undertaken did this patient demonstrate normalization of in vitro lymphocyte responses, appearance of delayed hypersensitivity skin test, and most importantly, sustained clinical remission..

The second patient, a 13-year old boy who originally presented with recurrent pulmonary infection and disseminated varicella, was found to have selective T-cell deficiency and intact B-cell function. Repeated courses of transfer factor not only resulted in skin test conversion but also in sustained clinical response. He is presently being maintained on transfer factor.

The third patient was a twenty-three year old female suffering from tuberculous peritonitis. Her disease, recalcitrant to conventional anti tuberculous therapy, was controlled only after therapeutic intervention with transfer factor. Despite this temporary reprieve, she succumbed last fall with gram negative sepsis.

In collaboration with the dermatology group, we have begun to study the efficacy of transfer factor in patients with anergy associated with severe eczema, a disease that has been associated with T-cell deficiency. To date, four patients have been treated with transfer factor with clinical improvement observed in two. One of four patients in the placebo group has likewise improved. Serial in vitro lymphocyte parameters have been followed concomitantly. This is an ongoing project.

Finally, transfer factor has been prepared for two patients with osteogenic sarcoma. These patients expired before evaluation of therapeutic efficacy could be ascertained.

Conclusions: Transfer factor therapy has demonstrated clear benefit in two patients with primary immunodeficiency diseases and one with an acquired defect. In addition, diagnosis, evaluation and treatment of these patients provide valuable training experience to allergy-immunology fellows and house staff.

Funds Utilized, FY-76: \$4,500

Funding Requirements:

<u>Personnel:</u>	One GS-7 technician 20 weeks.
<u>Equipment:</u>	No new equipment needed.
<u>Supplies:</u>	Consumable
<u>Travel:</u>	
<u>Mission:</u>	600.00
<u>Conference:</u>	650.00
	\$6,250.00

Publications: Ballow, M., and Good, R.A. Report of a patient with T-cell deficiency and normal B-cell function: A New Immunodeficiency Disease with Response to Transfer Factor, Cell. Immunol., 19, 219-229, 1975.

Type of Report: Interim-Renewal.

Date Prepared: 13 April 1976

Work Unit No.: 3103

Title of Project: Techniques for the Detection of Antinuclear Antibodies. Comparative Study -

Principal Investigator: Oliver J. Lawless, LTC, MC

Objectives:

- (1) To compare the 5 standard immunological techniques currently available for the detection of Antinuclear antibodies (ANA) in patients with Rheumatic disease processes.
- (2) To assess such techniques as regards.
  - (a) Sensitivity
  - (b) Specificity especially as regards differentiation of systemic lupus erythematosus (SLE) from Rheumatoid Arthritis (RA), scleroderma, polymyositis, juvenile Rheumatoid Arthritis, Mixed Connective Tissue diseases (M.C.T.D.), gout, and degenerative joint disease.
- (3) To ascertain the association between positive tests and clinical activity of disease.
- (4) To assess the reproducibility of the positive tests, and when titers of positivity and patterns of fluorescence are recorded.
- (5) To ascertain the association, if any, between patterns of fluorescence and (a) The DNA - DNP complement fixation test.  
(b) Radioactive C<sup>14</sup> DNA binding test.
- (6) To assess the ease and accuracy of standard ANA testing using KB tumour cells rather than mouse liver cells as a source of nuclei.
- (7) To analyze the cost and usefulness of the 5 methods of ANA detection.

Technical Approach: Sera from a panel of 150 patients of the Rheumatology and Clinical Immunology out-patient department rapidly frozen and stored at -70°C are being used. These sera are composed of known sera of patients with active and inactive SLE, RA, other connective tissue diseases, gout and degenerative joint disease. All sera were obtained for diagnostic purposes.

Sera will be randomly assigned a number and tested blindly by the investigator. Six techniques will be employed.

- (1) FANA - mouse liver nuclei by the method of Friou.<sup>3</sup>
- (2) FANA - KB tumour cells monolayer as source of nuclei.
- (3) Horse - Radish peroxidase test - This will be performed by Dr. Merrill Benson of Boston.
- (4) DNA - DNP complement fixation - This will be performed in the WRAIR laboratory under direction of Mr. Fife.
- (5) Latex "LE test" - This test commercially available will be performed according to the method proposed by the manufacturer (Lederle Laboratories).
- (6)  $\text{Cl}^{14}$  DNA antibody binding will be assayed by the modification of the Farr technique currently used in our laboratory.

Sera will initially be unfrozen, tested, and rapidly refrozen. The sera will again be unfrozen and retested. The sera will be tested for a third time following storage at 4°C for one week. Finally the sera will be tested following storage at room temperature for one week. The initial and second testing will be compared for reproducibility of each test. The third and fourth testing duplicates some of the common storage errors of sera sent for ANA testing and will be compared with the initial two test results.

Progress and Results: The first phase of this study comparing five different techniques for the detection of antinuclear antibodies had been completed last year and the data and conclusions are synopsised below.

Test	Controls	SLE	RA	JRA	PSS	PM
FANA-ML	4%	100%	31%	0%	35%	0%
FANA-KB	4	100	34	25	100	50
C.FIX. DNA	0	17	0	0	0	0
DNP	0	25	6	20	0	0
DNA BINDING	0	38	0	0	0	0
LATEX LE	0	20	9	0	0	0

Conclusions:

- (1) FANA-ML - or KB are the most sensitive tests for diagnosing SLE patients, but both are positive in a significant percentage of patients with other connective tissue diseases.

(2) DNA - Comp. fix. and DNA binding tests when positive are specific for SLE, but are less sensitive than FANA tests.

(3) A positive DNA binding test is not only specific for SLE, but is high when the disease is active, and normal or negative when in remission. With treatment the tests falls to negative range.

(4) A negative FANA, provided the patient is not on treatment rules out the diagnosis of SLE.

(5) Enough data is now available for standardization of the tests for SLE throughout the Army.

Because these results suggested specificity of the DNA binding test for SLE, a positive correlation with activity of disease and a possible correlation with nephritis, it was decided to use this test in a larger series of SLE patients with and without nephritis, with and without anti-inflammatory steroid and/or immunosuppressive drugs, in a serial fashion in order to see statistically:

(1) If the preliminary results are born out statistically in a larger sample.

(2) If successful treatment to abate clinical symptoms is associated with reduction in DNA binding results or not.

(3) If those patients treated successfully with and without steroids and/or immunosuppressive agents, (such as aspirin and chloroquine compounds) show a change in DNA binding results simultaneous with clinical improvement.

(4) If DNA binding tests have predictive value in regard to clinical relapses, and in regard to development of nephritis.

Summary of Results:

DNA binding tests have now been performed on approximately 485 positive FANA patients. Sera from 73 (15%) were positive with a binding level of 34%, -2 standard deviations above the mean of normal controls. Forty-four (44) of these patients have had 3 or more tests performed in a serial fashion at 1 to 2 monthly intervals. Duration of follow-up is variable from a few months to up to 3 years. Frequency of follow-up varies between weekly to 3 monthly intervals. Complete review and statistical analysis of these data has not been performed pending clinical

chart review. Preliminary analysis reveals the following results.

- (1) 18/44 (40%) of patients with a positive DNA binding test at time of presentation did not have clinical evidence of nephritis determined by active urinary sediment, proteinuria 600mg/24 hours, or impaired creatinine clearance.
- (2) Three patients with SLE and with positive DNA binding tests had clinical and serological criteria for mixed connective tissue disease.
- (3) Twenty-three (53%) of patients with positive DNA binding tests had clinical evidence of nephritis, 17 of which were confirmed by biopsy.
- (4) 9/10 patients initially treated with aspirin and hydroxychloroquinone compounds failed to show any change of DNA binding results within 1 to 2 months of treatment.

(5) Thirty-two (72%) were placed on steroids for clinical control of symptoms. In all but 4 of these, the DNA binding results fell within 1 month of commencing steroids, and this was associated with reduction in clinical symptoms. In 2 patients, however, there was either no change in DNA binding or symptoms (both patients were found not taking their medicines). In 1 patient, the DNA binding rose after commencing steroids and failed to normalize as did symptoms in response to steroids and azathioprine, one patient had an initial fall in DNA binding and improvement in symptoms followed by a plateau of DNA binding, above normal values and continued symptomatology. Both were unresponsive to increasing doses of prednisone and addition of cyclophosphamide.

Ten patients (22%) showed a relapse in clinical activity while undergoing reduction of steroid dose. In all patients the DNA binding tests were elevated again after an initial fall, and in retrospect could have predicted a return of clinical symptoms.

No firm conclusions can be drawn at this time pending chart review and statistical analysis of data. It would appear, however, that in general terms a positive DNA binding test may be present in patients without clinical evidence of nephritis; it is frequently positive when the disease is active clinically; non-steroidal anti-inflammatory agents do not alter DNA binding results; and that a reduction followed by a rise

in the test results may predict a flare in clinical activity of the diseases.

Funds Utilized FY 76:

Personnel: One GS-9 Step 3, Civilian Technician.  
2/5 times - \$6,206

Supplies: - 360

Funding Requirements, FY 77:

We request continued funding of this project as an essential component of the Rheumatology Clinical Immunology fellowship training program, and as an essential component of Project - 3123 which integrates humoral and cellular immunological mechanisms in the mechanism of the disease SLE.

We have been in contact with the Chief, Pathology, WRAMC, COL Hardman, and Chief, Immunology and Serology, WRAIR, LTC Diggs, with a view to formulating a policy for the handling of requests for antinuclear antibodies at WRAMC. Both sections are currently reviewing their work load and unit cost, as well as our data relating tests results to clinical disease and disease activity.

I feel that performance of the DNA binding test to double stranded DNA is mandatory for the diagnosis and treatment of patients with connective tissue diseases, and we request continued funding of this project to enable us to continue this technique for measuring antibodies to double stranded DNA, and to polydeoxyribonucleotides, Poly IC, Poly A and Poly U.

Personnel: One GS-9 Step 3, Civilian Technician.  
2/5 times - \$6,206

Equipment: No new equipment required.

Supplies: - 6,500

Travel: - 200

Reprints: - 150

Publications: None FY 1976.

Type of Report: Interim.

Work Unit No.: 3104

Title of Project: The Effect of Atropine and Salbutamol on Bronchial Response to Histamine.

Investigators:

Principal: Charlotte L. Casterline, MD

Associates: George W. Ward, Jr., COL MC  
Richard Evans, COL MC

Objectives: To demonstrate the effectiveness of aerosolized Salbutamol alone, and in combination with Atropine in blocking histamine-induced and meholyl-induced bronchospasm.

Technical Approach: Thirteen adult asthmatic patients were studied using the procedure recommended by the Standardization Panel of the Asthma Allergic Disease Centers, NIAID, for bronchial provocation. Solutions of meholyl and histamine were prepared for delivery by the dosimeter, measured dose delivery system. Forced lateral capacity, forced expiratory volume and peak flow were measured after each challenge. After baseline determinations, patients were given either Atropine in the dose of 5 mg of Atropine Sulfate for inhalation. A meholyl challenge was performed and when a decrease in FEV<sub>1</sub> did not occur, adequate blockade by Atropine was assumed and the histamine inhalation challenge was performed. Patients responding to bronchospasm with all challenges were reversed by means of isoprel aerosol.

Conclusions: The following conclusions can be drawn from this study:

1. Histamine induced bronchospasm in human asthmatics is reproducible by the technique of the dosimeter dose delivery system. Premedication with aerosolized atropine sulfate only minimally effects the bronchial response to histamine.
2. Pharmacologic blockade of the meholyl response was in evidence that sufficient large doses of Atropine had been administered.
3. The data has little or no support to the theory that aerosolized histamine acts primarily on the lung receptors to induce cholinergic mediated bronchial constriction.

Publications: This work has been submitted and accepted by the Journal of Allergy and Clinical Immunology for publication during 1976.

Type of Report: Final

Work Unit No.: 3105

Title of Project: An Evaluation of Immunologic Response in Ragweed-Sensitive Patients by New Techniques.

Investigators:

Principal: Richard Evans, COL MC

Associate: L. Blair Thrush, MAJ MC

Objectives: To intensively study the effects of high dose specific immunotherapy on extrinsic asthma. The goal is to document in an objective manner any change in sensitivity to an offending antigen.

Technical Approach: All patients have an extensive in vivo and in vitro evaluation prior to placement on immunotherapy. A detailed history and physical examination is preformed. Patients are selected because of relatively severe specific aero-allergen induced asthma. The following tests are done to assess each individual's degree of sensitivity to the offending antigen. The same antigen (i.e., company and lot number) was used for all testing and treatments.

In vivo studies:

- a) Serial skin test titration to extinction
- b) Antigen bronchial challenge

In vitro studies:

- a) Leukocyte histamine release (LHR)
- b) Total serum IgE
- c) RAST (specific IgE) -when technically possible.

After the above baseline studies, high dose specific immunotherapy is begun. The patients are followed clinically with repeat of all the above studies at 3 to 6 month intervals.

Progress and Results: Thus far five patients have entered the study. The first three are West Point cadets each with marked Timothy grass sensitivity. They are six to nine months into the study. All are doing well clinically.

Initially at three or six months they showed an increased sensitivity by LHR with no significant change in their skin or bronchial sensitivity. This is not unexpected based on prior work suggesting that immunotherapy with ragweed produces first an increased basophil sensitivity (similar to that seen naturally during the ragweed season). On long term therapy hopefully we will see decreased sensitivity in LHR as well as bronchial sensitivity.

The other two patients are Army behavioral psychologists (PhD's) who had marked rat dander sensitivity. As their careers depend on significant dander exposure, the decision was made to attempt immunotherapy using an extract made from their particular rats (the Sprague-Dolly species). This was done by the Allergen Extract Laboratory of Walter Reed Army Medical Center. The first patient has completed the first year of therapy. Clinically his asthma and allergic rhinitis have improved.

His LHR has shown an increase in basophil sensitivity, however, his skin sensitivity, total IgE and bronchial sensitivity have decreased. The second patient has just entered the study and is tolerating his rat dander immunotherapy without difficulty.

**Conclusions:** This protocol warrants continuation. It has been productive of two publications and four scientific presentations. It is extremely valuable in our fellow teaching program.

Funds Utilized FY-76: \$7,650 and GS-07 Technician/36 weeks

Funds Required FY-77:

Personnel: One GS-07 Technician 36 weeks per year

Equipment: None

Supplies: Consumable supplies \$7,000

Travel: Mission 400  
Conference 700

Publications: 400

Total \$8,500

Funds Required FY-78: Same as FY-77

Publications:

1. Richard Evans, Hobert Pence, Hyman Kaplan and Ross E. Rocklin, The Effect of Immunotherapy on Humoral and Cellular Responses in Ragweed Hayfever. The Journal of Clinical Investigation, 57:1378-1385, May 1976.
2. Richard Evans, The Radioallergosorbent Test (RAST) as a Research Tool, RAST Symposium, Palm Springs, California, 1975.

Type of Report: Interim

Work Unit No.: 3106

Title of Project: Immunologic Aspects of Lung Disease

Principal Investigator: George W. Ward, Jr., COL MC

Objectives: The purpose of this study is to select patients with pulmonary diseases of obscure etiology and by specific immunological techniques, attempt to clarify the pathogenesis, etiology, or pathogenetic mechanism of these diseases. The techniques used are: light and electron microscopy, fluorescent antibody staining, and evaluation of possible production of MIF by sensitized lymphocytes.

Technical Approach: Tissue specimens obtained from diagnostic fiberoptic bronchoscopy procedures were examined utilizing direct and indirect immunofluorescent techniques. These specimens were examined for the presence of immunoglobulins, G, A, M, D and E, and for the presence of complement components. After the specimens were examined the findings under immunofluorescence were correlated. An antisera to pigeon antigen was prepared in rabbits and using a double layer technique, a patient with pigeon breeder disease was shown to have specific antibody activity to the antigen contained in pigeon serum.

Progress and Results: A total of 486 specimens have been examined by means of immunofluorescent techniques in this protocol. The data was presented to the American Academy of Allergy in their annual meeting in 1976. The data has also been compiled into a manuscript which is under final preparation for submission to the Journal of Allergy and Clinical Immunology.

Type of Report: Final

Work Unit No.: 3107

Title of Project: Culture, Identification, and Extract Preparation of Thermophilic Fungi Involved in Hypersensitivity Pneumonitis.

Principal Investigator: George W. Ward, Jr.

Objectives: To be able to recover by culture, and then identify and make extract preparations of various thermophilic fungal organisms for in vitro immunoprecipitin testing of patients with various hypersensitivity pneumonitis type syndrome.

Progress and Results: These organisms have proven to be exceedingly difficult to culture. Furthermore, control cultures for comparison are required for specific identification. Fortunately, we have been provided by these control cultures by Dr. Jordan Fink of Wisconsin. Greer Laboratories has been helpful in establishing a technique for thermophilic cultures. Six cultures of thermophilic organisms from patients' home and work environments, have been prepared in the past year. These cultures were helpful in the evaluation of the patients' clinical disease state manifesting in hypersensitivity pneumonitis.

The Principal Investigator has transferred from Walter Reed Army Medical Center and this protocol will be discontinued.

Publication: A summary of the project was presented to the Pulmonary Allergy Symposium, Fitzsimons Army Hospital, September 1974.

Type of Report: Final

Work Unit No.: 3109

Title of Project: Complement Deficiencies and their Relationship to Disease in Man.

Investigators:

Principal: Arnold Levinson, M.D. MAJ MC

Associate: Richard Evans, III, M.D. LTC MC

Objective: The complement system, a series of interacting proteins, plays an integral role in the inflammatory response. It is clear that an intact complement system is indispensable in the defense against infections. In other instances activation of the complement system, with generation of phlogistic mediators, may have detrimental consequences for the host. This is an ongoing project in which we are investigating not only the integrity of the complement system in patients with recurrent infection but also the active participation of this system in various disease states.

Technical Approach: Our laboratory has the capability of measuring several complement components immunochemically including the first complement component C1q, the fourth complement component, C4 third complement component, C3 and a component of the alternate complement pathway known as C3 pro-activator or properdin Factor B. C3 and C4 are measured from material purchased from commercial sources. C1q and C3 pro-activator are measured by immunochemical plates which are made in the laboratory with specific anti-sera from commercial sources.

Progress and Results: Mark Ballow, M.D. MAJ MC has departed from Walter Reed Army Medical Center and the new principal is Arnold Levinson, M.D. MAJ MC.

1. We have continued our studies on the complement abnormalities in chronic urticaria and/or angioedema. Using the complement profile as a probe, we have begun to elucidate the pathogenesis of this problem in some patients who heretofore have been classified as having idiopathic urticaria. Of 103 patients previously investigated, a novel form of complement activation was identified. This work was recently published in Lancet (see below). To date follow-up of these patients indicates that they are free of collagen vascular or other diseases associated with complement activation. Of thirty-four additional individuals studied, five have had reductions in C3. Like the original patients, they too have had normal C4 values, suggesting that a pathway other than the classical one is involved. Whether this is indeed the case will hopefully be answered in the near future. It should be mentioned that, following our lead, other groups are now beginning to report complement abnormalities in similar patients. Many of the chronic angioedema patients have been referred for us to rule out the diagnosis of hereditary angioedema. Based on serum C4 determinations, we have been able to substantiate or rule out this diagnosis.

2. We have continued our studies of the complement system in patients with recurrent infections. A 4-1/2 year old girl, who had life-long infections with encapsulated bacteria and gram negative organisms, was found to have a complete absence of total hemolytic complement and C<sub>3</sub>. This deficiency had been previously reported in only one other patient, and is the subject of a recent paper in the Journal of Clinical Investigation (see below). Currently we are investigating a 2-year old boy with recurrent otitis media who was found to have depressed serum C<sub>3</sub>, as well as two brothers with a similar problem.

3. In collaboration with the renal and transplant services, we have examined serum for complement abnormalities in 52 individuals with a variety of kidney diseases. Reduced serum complement values, particularly C<sub>3</sub> and C<sub>4</sub>, have been found at some time in the course of patients suffering from suspected immune mediated kidney disease, e.g., systemic lupus erythematosus, acute poststreptococcal glomerulonephritis, etc. Moreover, these reductions correlate extremely well with clinical disease activity and provide an objective basis for therapeutic decisions. In the near future we expect to study transplant patients prospectively, pre and post transplant, looking for evidence of complement activation in rejection.

Conclusion: Our complement laboratory has continued to provide useful and necessary information in the management of an heterogeneous group of diseases. In addition, we have expanded our studies on a novel form of urticaria and added the second case of hereditary C<sub>3</sub> deficiency to the world's literature. Projected studies will further delineate the role of serum complement in transplant rejection. Finally, patients identified as having complement abnormalities provided valuable teaching devices for our fellows and house staff.

Funds Utilized, FY-76: \$4,000

Funding Requirements, FY-77:

<u>Personnel:</u>	One GS-7 technician, currently employed, 20 weeks/year.
<u>Equipment:</u>	No new equipment is required.
<u>Supplies:</u>	Consumable supplies \$4,500.00
<u>Travel:</u>	
<u>Mission:</u>	400.00
<u>Conference:</u>	600.00
	<hr/>
	\$5,500.00

Publications:

1. Ballow, M., Shira, J.E., Larden, L., Yang, S.Y., and Day, N.K.: Complete Absence of the Third Component of Complement in Man. J. Clin. Invest. 703, 1976.
2. Ballow, M., Ward, G.W., Gershwin, M.E., and Day, N.K.: Cl-Bypass Complement-activation Pathway in Patients with Chronic Urticaria and Angioedema. Lancet, 248-250, 1975.

Type of Report: Interim-Renewal.

Work Unit No.: 3110

Title: The Detection of Anti-Skin Antibodies in  
Sera and Skin Biopsies of Patients with  
Pemphigus, Pemphigoid, and Lupus  
Erythematosus.

Principal Investigator: Major Robert A. Davis, MD  
Department of Medicine  
Dermatology Service

Status: Several requests for the Annual Progress  
Report on this project have been ignored.  
The Clinical Investigation Committee in a  
meeting on 29 September 1976, terminated  
this study.

Work Unit No.: 3111

Title of Project: Quantitative Serum IgE in Human Infections, Immune Deficiency States and Diseases with Impaired Cellular Immunity.

Investigators:

Principal: Richard Evans, LTC MC

Associate: Virginia Battista, BA

Objectives: To study the role of IgE responses in non-atopic states with emphasis on a possible association between elevated or decreased levels of serum IgE and human infections, human immune deficiency states, and impaired cellular immune responses.

Technical Approach: Serum samples from a patient population described above are collected. These are then analyzed for IgE content using a radioimmunoassay. Reagents for this assay (the Phadebas Test) are purchased from Pharmacia Laboratory. The mean IgE level in our laboratory with normal sera has been 138 I.U./ml with a standard deviation of 113 I.U./ml.

Progress & Results: In the past year we have measured quantitative serum IgE in 774 serum samples. Clinical investigation protocols which have included measurement of serum IgE are: Immunologic Aspects of Lung Disease, In Vitro Immunologic Change Induced by Immunotherapy in Deerfly Patients, Hypersensitivity by Reactions in Humans Allergic to Bee Sting, and Immunologic Evaluation of Immune Deficiency Disease States.

Serum IgE determinations have also been performed in support of clinical patient evaluations such as: eosinophilia of undetermined origin, pulmonary allergic aspergillosis, Bakiso asthma, laboratory workers asthma, and insulin allergy.

We are also measuring serum IgE for the WRAMC Blood Bank in all instances of transfusion reaction. We have performed IgE determinations in patient sera at special request for George Washington University, Howard University, Portsmouth Naval Hospital, and Bethesda Naval Hospital. The number of these special requests is not unreasonable, and our cooperation enhances the professional relationship of the institutions.

The Principal Investigator was recently (Mar 76) made chairman of a new committee on Standardization of In Vitro Tests in Allergy, of the American Academy of Allergy Quantitation of IgE is the first test under view. This will be a new research effort of this protocol.

Conclusions: The protocol warrants continuation for another year. The protocol represents an on-going study which supports the house staff teaching mission of WRAMC as well as the mission of clinical research.

Funding:

Utilized FY-76:

Personnel: One GS-07 Technician, 9 weeks/per year

Supplies: \$6,800 (first three quarters)

Requested FY-77

Personnel: One GS-07 Technician, 9 weeks/per year. Technician is currently available in the Clinical Investigation Service.

Supplies: Radioimmunoassay Materials \$9,100.

Publication:

Quantitative Immunoglobulin Levels in Tuberculosis. Charlotte L. Casterline, MD, Richard Evans, LTC MC, and George W. Ward, Jr., MD. CHEST, (in press).

Type of Report: Interim

Work Unit No.: 3112

Title of Project: Lymphocyte Subpopulation Identification

Investigator:

Principal: Oliver J. Lawless, MD, LTC, MC

Objective: Lymphocytes in human peripheral blood and synovial fluid represent aggregations of at least two subpopulations. One subpopulation spontaneously forms rosettes with sheep red blood cells and probably represents thymus derived cells (T cells). A second subpopulation shows surface immunoglobulin staining and probably represents bone marrow derived cells, (B cells).

Progress and Results: No further results have been accomplished on this protocol in regard to connective tissue diseases. The reasons for not pursuing it further were: (1) reports were already well documented in the literature on T & B Cell differentiation in rheumatoid arthritis and systemic lupus erythematosus; (2) our results were in agreement with results already reported and which were summarized in last years annual progress report; (3) the remaining diseases seen in Rheumatology and Clinical Immunology Clinic have less evidence to support dominant immune etiology being responsible for their causation and are seen in numbers too low to generate statistically significant results.

Conclusions: N/A

Funds Utilized FY-76: None

Funding Requested FY-77: None

Publications FY-76: None

Type of Report: Terminated

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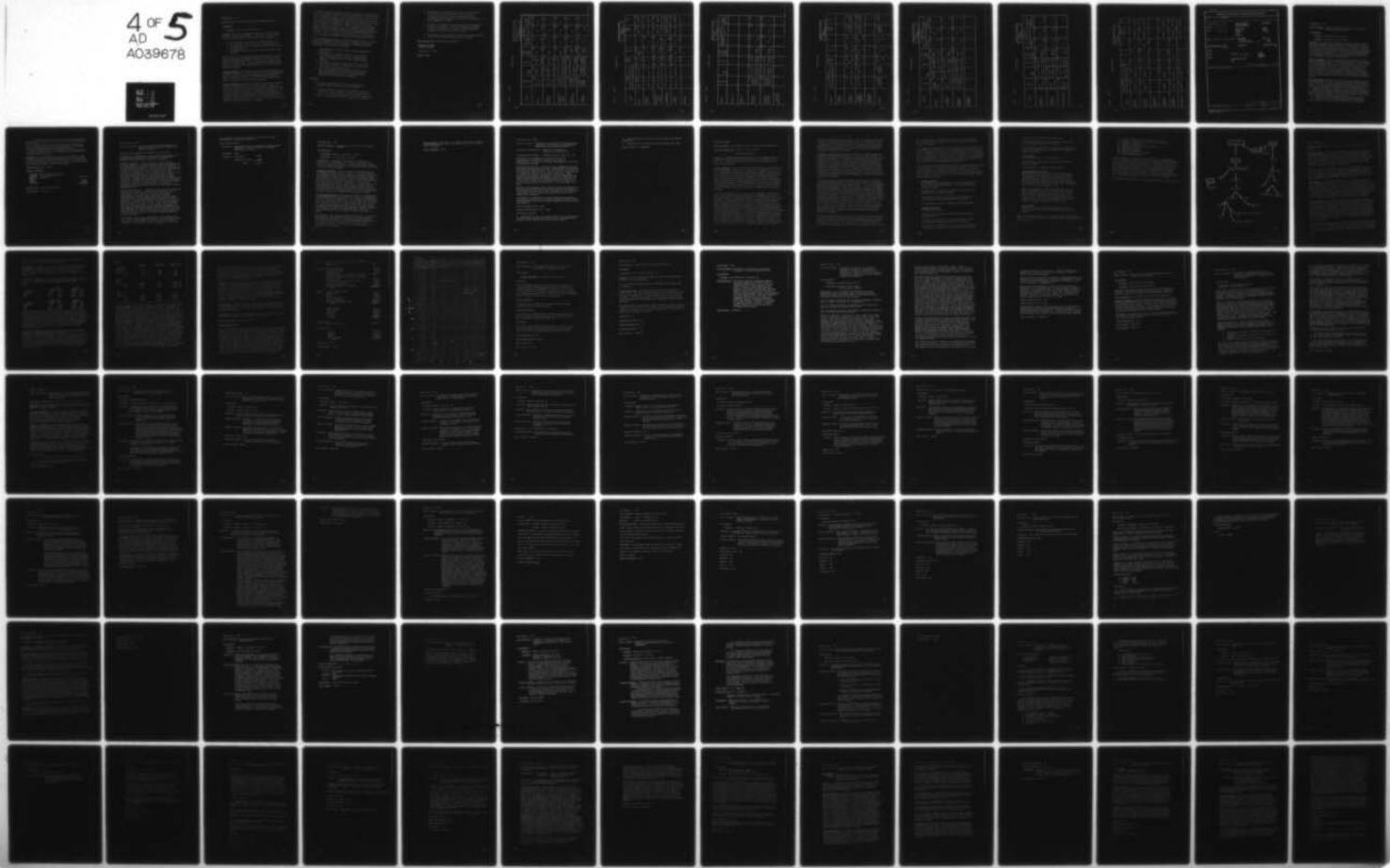
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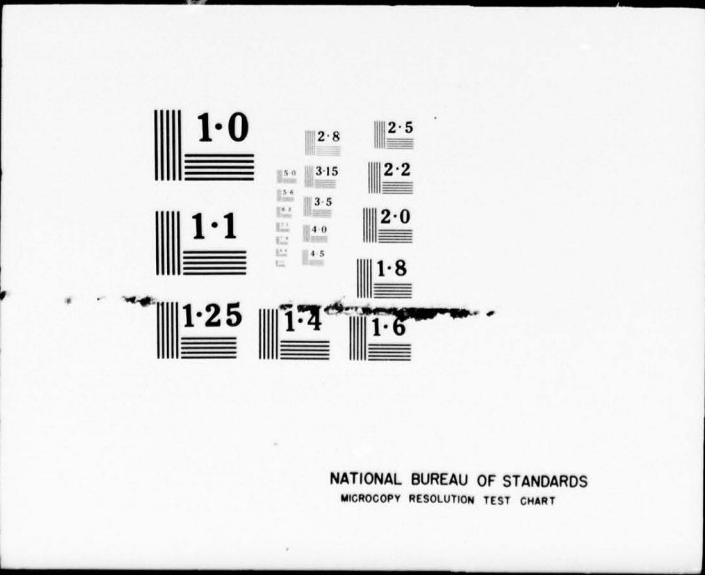
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Work Unit No.: 3113

Title of Project: Synovial Fluid Analysis in Rheumatic Disease

Investigator:

Principal: Oliver J. Lawless, MD, LTC, MC

Objective: Patients with rheumatic diseases often present initially and recurrently with joint effusions. Classically, synovial fluid analysis has differentiated the fluids into 4 gross categories.

- 1) The low white count, good mucin clot type found in D.J.D.
- 2) The higher white count, poor mucin clot type found in R.A.
- 3) The higher white count associated with crystals found in gout and pseudogout, and
- 4) The higher white count, low glucose type seen with bacterial infection.

At this time it is clear that such broad classifications have limited value in diagnostic and management situations. The advent of more sophisticated immunological tools lends itself to the further classification of synovial fluids. It is proposed that all patients being diagnostically evaluated as regard synovial fluid will have the fluid analyzed per protocol.

Technical Approach: This study will help in the current diagnosis and management of patients in Walter Reed Army Medical Center and those seen in the Rheumatology and Clinical Immunology Outpatient Department of WRAMC. Valuable information will be tabulated for publication when sufficient numbers of synovial fluids are analyzed.

Progress and Results: Synovial fluid analysis has now been carried out on several hundred samples of joint fluid. Complete clinical and laboratory data were available on 225 patient samples, which fall into 16 different categorical causes for or diagnosis of "arthritis". These results are listed in Table form. The fluid was analyzed for the following parameters: Turbidity, Mucin Clot, Viscosity, Leukocyte Count and differential, Presence of Crystals, Total Protein, Glucose, C<sub>3</sub> complement, Rheumatoid factor, and FANA tests. An example of the routine worksheet listing these parameters is appended.

In certain circumstances enough fluid was not available to perform all tests. In these circumstances, the data can be interpreted by the following code as used in the Tables appended. In the top right hand corner of each box the number in parenthesis represents the total number of fluids tested. In the left top corner the minimum value, in the center, the average, and the lower right hand corner the maximum value recorded for that particular test. The range of diagnosis were as follows: Normals, Traumatic Arthritis, Degenerative Joint Disease, Class I; Lupus, Gout, Rheumatic Fever, Rheumatoid Arthritis, Juvenile Rheumatoid Arthritis, Ankylosing Spondylitis, Sjogren's Syndrome, Colitis, Class II; Septic Class III; and Hemmorrhagic-Class IV holds true. It is to be noted, however, that there is a definite overlap in many of these broad categories and this study documents such overlap.

It further emphasizes that proper synovial fluid analyses may not of itself establish the diagnosis, however, in most circumstances it narrows the differential diagnosis to two broad categories, inflammatory and non-inflammatory. Of interest are the findings:

- 1) Inflammatory type fluids in certain clinically degenerative type joints,
- 2) The importance of correlating synovial fluid complement levels with protein levels to reduce the possibility of using a low complement level to infer on immune mediated arthritis,
- 3) The significant incidence of FANA positivity in the fluids of RA and JRA in addition to SLE, and of RF positivity in SLE in addition to RA and JRA, and,
- 4) The lowest complement level we found when related to protein concentration, was in a case of colitic arthritis rather than in SLE or RA, and in contrast consistently high values were found in Reiter's Syndrome.

Conclusions:

- 1) No single test such as total white count is of value in establishing the diagnosis of a type of arthritis.
- 2) A combination of tests as used in this study may likewise not establish the cause of arthritis, however, it narrows it down to one of a few differential diagnosis which taken together with the clinical setting can invariable establish the diagnosis.

- 3) Total white count — 50,000 while typical of septic arthritis, can be present in other diseases, eg., crystal induced synovitis, JRA, RA, and Reiter's, and mandates the use of polarizing microscope, appropriate cultures, and other tests to avoid inappropriate treatment.
- 4) Serologies and complement levels while they are never diagnostic and have a low incidence of positivity, may produce important clues to the consideration of Connective Tissue Diseases not considered on clinical grounds to be pertinent.
- 5) Larger number of fluid analysis are required in several categories in order to produce statistically relevant comparisons.

Funds Utilized FY 76: \$925.00, Funded \$1,605.00

Funding Requirements:

Supplies - \$925.00

Publications - \$0.00

Report: Interim

TABLE I

PAGE 1

## SYNOVIAL FLUID

MINIMUM (# of Fluids)		
Average		
Maximum		

DISEASE	Turbidity	Mucin Clot	Viscosity	Total Leukocytes per cu. mm.	CELL TYPE %		
					Polymorpho-nuclears	Lymphocytes	Mono Nuclear phagocytes
NORMAL	(46) 1+ - 100%	(25)	(22)	(29) 0 - Low	29	6	(29) 0 (29)
	2+ - 0	0 - Poor	0 - Fair	63 0 - Fair	7	25	63
	3+ - 0			180 100%-High	25	78	71
	4+ - 0	100%-Good					
TRAUMATIC ARTHRITIS	(1) 1+ - 0	(2)	-	(1) 34 0 - Poor	(2) 34	30	(2) 0 (2)
	2+ - 0	0 - Fair	100%-Fair	4,500 100%-Fair	52	48	0
	3+ - 0	0 - Good	0 - High	-	70	66	0
	4+ - 0						
DEGENERATIVE JOINT DISEASE	(47) 1+ - 72.4%	(50) 4.0%-Poor	19.4%-Low	(51) 0 5.6%-Fair	(43) 0	(43) 5	(43) 5 (43)
	2+ - 23.4%			1,957 5.6%-Fair	27	70	1
	3+ - 2.1%	20.0%-Fair	75.0%-High	22,800 75.0%-High	95	100	11
	4+ - 2.1%	76.0%-Good					
LUPUS ERYTHEMATOSUS	(7) 1+ - 14.3%	(7) 57.1%-Poor	75.0%-Low	(10) 0 0 - Fair	(9) 1	(9) 0 (9)	(9) 0 (9)
	2+ - 28.6%	42.9%-Fair	25.0%-High	14,269 32,000	62	38	3
	3+ - 28.6%	0 - Good			99	100	5
	4+ - 28.6%						
GOUTY ARTHRITIS	1+-25% (4)	(4) 75.0%-Poor	33.3%-Low	(6) 1,440 33.3%-Fair	2	(6) 0 (6)	(6) 0 (6)
	2+-25%	0 - Fair	33.3%-Fair	77 17,083	7	7	0
	3+-25%	25.0%-Good	33.3%-High	42,300	93	23	0
	4+-25%				98		

TABLE I PAGE 2

## SYNOVIAL FLUID

MINIMUM (# of Fluids)  
Average  
Maximum

DISEASE	CRYSTALS		TOTAL Protein	Glucose	$C_3$	Rheum. Factor.	FANA
	+	-					
NORMAL	(46)	(46)	1.1 (10) 1.7 2.1	(46) Approx. Eq.	-	(46) 100% - Neg	(46) 100% - Neg
TRAUMATIC ARTHRITIS	(2)	(2)	- (1)	- (1)	- (1)	(2)	(2)
DEGENERATIVE JOINT DISEASE	(39)	(39)	1.5 (22) 3.0 5.7	63 (25) 95 153	21 (25) 38.1 69	100% - Neg (34)	100% - Neg (29)
LUPUS ERYTHEMATOSUS	100% - Neg	100% - Neg	3.7 (3) 4.2	52 (2) 70	30 (5) 60.6 88	71.1% - Neg 28.6% - Pos 88	60.0% - Neg 40.0% - Pos (7)
GOUTY ARTHRITIS	100% - Pos	100% - Pos	4.3 (2)	98 (2) 99 4.9	65 (2) 84.5 100	100% - Neg (4) 104	100% - Neg (2)
PSEUDOGOUT	-	-	-	-	-	-	-

TABLE I PAGE 3

## SYNOVIAL FLUID

MINIMUM (# of Fluids)
Average
Maximum

DISEASE	Turbidity	Mucin Clot	Viscosity	Total Leukocytes per cu. mm	CELL TYPE %		
					Polymorpho-nuclears	Lymphocytes	Mono Nuclear Phagocytes
PSEUDOGOUT	-	-	-	-	-	-	-
RHEUMATIC FEVER	-	-	-	-	-	-	-
RHEUMATOID ARTHRITIS	(50) 1+-10.4% 2+-43.8% 3+-22.9% 4+-25.0%	(53) 65.9%-Poor 23.4%-Fair 10.6%-Good	(49) 70.8%-Low 16.7%-Fair 12.5%-High	325 12,696 36,000	(57) 10 73 100	(52) 0 25 89	(52) 0 2 16
JUVENILE RHEUMATOID ARTHRITIS	(16) 1+-12.5% 2+-25.0% 3+-37.5% 4+-25.0%	(11) 36.4%-Poor 54.5%-Fair 9.1%-Good	(13) 84.6%-Low 10.4%-Fair 5.0%-High	(21) 30,808 74,600	(19) 8 74.6 95	(19) 0 24.5 92	(19) 0 5 10
ANKYLOSING SPONDYLITIS	-	-	-	-	-	-	-

TABLE I PAGE 4

## SYNOVIAL FLUID

MINIMUM (# of Fluids)		
Average		Maximum

DISEASE	CRYSTALS		TOTAL Protein	Glucose	$\text{C}_3$	Rheum. Factor	FANA
	+	-					
RHEUMATIC FEVER	-	-	-	-	-	-	-
RHEUMATOID ARTHRITIS	(41) 100% - Neg	(41) 100% - Neg	2.9 4.8 6.8	55 89.7 121	21 61.0 136	(36) 67.5% - Neg 32.5% - Pos	(40) 88.9% - Neg 11.1% - Pos
JUVENILE RHEUMATOID ARTHRITIS	(7) 100% - Neg	(7) 100% - Neg	3.8 4.6	53 89.2	50 95.2	(15) 76.9% - Neg 23.1% - Pos	(13) 91.7% - Neg 8.3% - Pos
ANKYLOSING SPONDYLITIS	-	-	-	-	-	-	-
SJOGREN	(5) 75.0% - Neg 25.0% - Pos	(5) 75.0% - Neg 25.0% - Pos	4.2 5.0	66 84	(2) 64.3	(4) 20.0% - Neg 87	(5) 80.0% - Neg 20.0% - Pos

## SYNOVIAL FLUID

MINIMUM (# of Fluids)

Average

Maximum

DISEASE	Turbidity	Mucin Clot	Viscosity	Total Leukocytes per cu. mm.	CELL TYPE %		
					Polymorpho-nuclears	Lymphocytes	Mono Nuclear phagocytes
SJOGREN	(2) 1+-0 2+-100% 3+-0 4+-0	(2) 100%-Poor 0 -Fair	(3) 75.0%-Low 25.0%-Fair	(4) 1,459 5,362	14 48	10 44.8	(6) 0
		- Good	0 - High	13,200	90	86	0
		(2) 100%-Poor	100%- Low	4,450 13,275 22,000	(2) - 90	(2) - - 10	(1) 0 -
		0 - Fair 0 - Good	0 - Fair 0 - High				(1) 0 0
COLITIC ARTHRITIS	(2) 1+-0 2+-0 3+-0 4+-100%	(2) 100%-Poor	100%- Low	560 13,396 35,700	(10) 30 91	(9) 82 91	(9) 0 70
		0 - Fair 0 - Good	0 - Fair 0 - High				
		(8) 1+-11.1% 2+-11.1% 3+-33.3% 4+-33.3%	(9) 44.4% - Poor 33.3% - Fair 22.3% - Good	560 13,396 35,700	(10) 30 91	(9) 82 91	(9) 0 70
		-	-			-	-
TUBERCULOUS ARTHRITIS	-	-	-			-	-

TABLE I

PAGE 6

## SYNOVIAL FLUID

MINIMUM (# of Fluids)		
Average		
Maximum		

DISEASE	Turbidity	Metin C lot	Viscosity	Total Leukocytes per cu. mm.	CELL TYPE %	
					Polymorpho-nuclears	Lymphocytes
GONOCOCAL ARTHRITIS	(4)	(4)	(4)	1,450 (4)	50 (4)	0 (4)
	1+ - 0	50% - Poor	100% - Low	22,612	83	18
	2+ - 0	50% - Fair	0 - Fair	43,000	100	50
	3+ - 0	0 - Good	0 - High			0
INFECTIOUS ARTHRITIS	4+ - 100%					0
	(4)	(4)	(4)	(6)	30 (5)	1 (5)
	1+ - 0	75.0% - Poor	50% - Low	100,150	83	17
	2+ - 0	25.0% - Fair	25% - Fair	260,000	99	70
CHONDROMALACIA	3+ - 0	0 - Good	25% - High			0
	4+ - 100%					0
	(2)	(2)	(2)	256 (4)	- (1)	- (1)
	1+ - 0	0 - Poor	0% - Low		0	0
	2+ - 50	0 - Fair	50% - Fair	740	99	1
	3+ - 50	100 - Good	50% - High	2,000	-	-
	4+ - 0				0	0

TABLE I PAGE 7

## SYNOVIAL FLUID

MINIMUM (# of Fluids)		
Average		
Maximum		

DISEASE	CRYSTALS		TOTAL Protein	Glucose	$\text{C}_3$	Rheum. Factor	FANA
	+	-					
COLITIC ARTHRITIS	(2)	(2)	(1)	(1)	-	(1)	(2)
	100%--Neg	100%--Neg	9.4	11.4	-	37	100%--Neg
REITER'S SYNDROME	(8)	(8)	3.4	60	(7)	84	(8)
	100%--Neg	100%--Neg	6.5	85.7	120.6	120.6	100%--Neg
TUBERCULOUS ARTHRITIS	-	-	14.5	100	193	-	-
GONOCOCCAL ARTHRITIS	(4)	(4)	3.3	(4)	6	(4)	(4)
	100%--Neg	100%--Neg	4.6	11	-	100%--Neg	100%--Neg
INFECTIOUS ARTHRITIS	(4)	(4)	-	(1)	-	(1)	(2)
	100%--Neg	100%--Neg	50	40	-	91	100%--Neg
CHONDROMALACIA	(2)	(2)	-	(1)	-	-	(4)
	100%--Neg	100%--Neg	3.3	96	-	-	100%--Neg

TABLE II

RHEUMATOLOGY AND CLINICAL IMMUNOLOGY SYNOVIANALYSIS CHART					
DATE:	DIAGNOSIS:				
FLUID FILED	<input type="checkbox"/> yes	<input type="checkbox"/> no	JOINT ASPIRATED: <u>VOLUME ASPIRATED:</u>	<u>MUCIN TEST</u>	
FASTING	<input type="checkbox"/> yes	<input type="checkbox"/> no	APPEARANCE	<u>SUGAR</u>	
BIOPSY	<input type="checkbox"/> yes	<input type="checkbox"/> no	COLOR: TURBIDITY: CLOT: VISCOSITY:	<u>BLOOD:</u> <u>S. FLUID:</u>	
<u>CELLS:</u> WCC	POLYS	LYMPHS	MONOS	<u>INCLUSIONS</u>	<u>RCC:</u> Few Many TNTC
				# Cells With _____ # Inclusions per Cell _____	
<u>CULTURE BACTERIAL</u> Aerobic Anaerobic			<u>GRAM STAIN</u>		<u>COMPLEMENT:</u> BIC CH50
<u>FUNGAL</u>					
<u>VIRAL</u>	<u>CRYSTALS</u>			<input type="checkbox"/> yes Bire <input type="checkbox"/> no Bire	<u>LIPIDS</u> <u>ENZYMES</u> <u>PROTEIN</u>
<u>OTHER</u>	<u>RHEUMATOID FACTOR</u> <u>FAT</u>				
_____ (Signature) M.D.					
COMMENT AND CLINICAL INTERPRETATION:					
_____ (Signature) M.D.					
PATIENT'S NAME (Last, First, Middle):			SSAN:	STATUS:	
				277	

Work Unit No.: 3117

Title of Project: Evaluation and Study of Patients with Primary and Secondary Immunodeficiencies

Investigators:

Principal: Arnold Levinson, M.D. MAJ MC

Associate: Richard Evans III, M.D. LTC MC

Objective: The immune system consists of a number of complex cellular and humoral components acting to protect the intact organism. When the integrity of this system is breached, either due to genetic aberrations or to acquired disease, individuals suffer dire consequences. The present protocol has been developed to identify and further the understanding of such breaches of host defenses. The elucidation of pathogenic mechanism in immune deficiencies is requisite for therapeutic intervention. In addition study of these so-called experiments of nature often leads to new insights into the immune response itself.

Technical Approach: The Clinical Immunology Investigative Laboratory is able to study a broad range of immune functions, relevant to both major arms of the immune response, i.e., T and B cell systems. It is becoming increasingly apparent that this kind of capability is necessary in a major teaching and referral center. We continue to employ all of the techniques mentioned in last year's annual report. In addition it should be mentioned that we have modified our basic cell culture technique so that smaller samples of blood are obtained from our patients. Also we are in the process of setting up a leukocyte migration inhibition assay to further expand our battery of tests for evaluating cell mediated immunity.

Progress and Results: Within the past year we have examined the cellular and humoral immune function of over fifty individuals. These patients have been referred by various departments within Walter Reed Army Medical Center as well as armed forces facilities throughout the world. For the most part such individuals have presented with recurrent infections.

To date our studies have provided not only the diagnosis but also the most appropriate therapeutic strategies in a number of these individuals. In addition we have identified and are beginning to unravel immune deficiency states which heretofore were either not described or poorly understood. Listed below are some of these patients:

1. Two individuals with common variable hypogammaglobulinemia
2. A baby with a newly characterized syndrome, Pseudo-DiGeorge Syndrome, which we are in the process of submitting for publication

3. A family with several members who have dysgammaglobulinemia in association with complement deficiency. This entity has previously not been reported.

4. A family in which two siblings had common variable hypogammaglobulinemia and associated interstitial lung disease. Other family members have autoimmune disorders and complement defects.

Certainly just as important as the delineation of new syndromes, is the assurance we provide to referring physicians and families when our extensive workups are negative.

Conclusions: Our immunodeficiency protocol plays a vital role in the delineation of aberrant immune mechanisms in a diverse array of disease states. Not only have our studies provided the means for diagnosis but also intelligent selection of therapy. In addition we are in the process of described several novel immune deficiency states.

Funds Utilized, FY-76: \$5,525

Funding Requirements:

<u>Personnel:</u>	One GS-7 technician 30 weeks	
<u>Equipment:</u>	No new equipment needed	
<u>Supplies:</u>	Consumable	\$5,525.00
<u>Travel:</u>		
<u>Mission:</u>		600.00
<u>Conference:</u>		650.00
		<u>\$6,775.00</u>

Type of Report: Annual Progress-Interim

Date Prepared: 26 May 1976

Work Unit No.: 3119

Title of Project: The Role of Sensitized Leukocytes in Antigen Induced Serotonin Release from Human Platelets.

Principal Investigator: Richard Evans, Colonel MC

Objectives: To demonstrate the presence (or absence) of a platelet activating factor released by IgE mediated, immediate hypersensitivity reactions of humans.

Technical Approach: A homocytotropic (IgE) antibody mediated response in human leukocytes has been demonstrated. As a consequence of antigen challenge, sensitized human basophils release histamine. The presence (or absence) of a factor released by these sensitized cells and which affects a platelet response was first demonstrated in human leukocyte preparations by Dr. Benveniste, NIH, Fed Pro 1974. The indicator system used by Dr. Benveniste was rabbit platelet histamine release. Human platelets contain serotonin. A method for demonstrating active absorption and release of serotonin by human platelets has been developed. This method provides an opportunity to evaluate the possible release of platelet activating factor from antigen stimulated sensitized human leukocytes, and which activates human platelets.

Progress and Results: Progress to date has been promising. Recent experiments were performed with Hypaque Ficoll differential centrifugation and glass bead column chromatography, to get better controlled basophil preparations. Moore's Methyl, Toluidine, Blue, Basophil stain has shown such preparations to contain up to 20% basophils. The previously described C<sup>14</sup> serotonin platelet labelling system is still in use. Experiments attempted thus far include platelet release using 1 ml supernatant fractions of ragweed antigen E challenged basophils; and dose-response experiments using different concentrations of the supernatants. Experimental results indicate that the supernatants may indeed contain platelet activating factor. Further experiments will be directed toward reproducing previous results.

Conclusions: This is a basic science oriented protocol which will require supply expenditures of less than \$1,000. The concept is an important one and a yes-no answer is possible. The protocol warrants completion. The project

has provided a pre-medical student in the summer program with excellent laboratory experience.

Funding Requirements:

Personnel: GS-04 Health Aide, currently a student summer employee in our Investigative Immunology Laboratory, 3 months.

Equipment: None

Supplies: Estimated \$900

Publication costs 250

Total \$1,150

Work Unit No: 3120

Title of Project: Precipitating Antibodies in Clinical Disease.

Investigators:

Principal: George W. Ward, Jr., COL MC

Associate: Ms. Tami Hase, BS, DAC

Objectives: To evaluate the presence or absence of precipitating antibodies in the sera of patients with a variety of clinical diseases, both pulmonary and systemic and to correlate the presence or absence of these antibodies with the clinical disease.

Technical Approach: In the diseases of hypersensitivity pneumonitis, precipitating antibodies have been shown to be of diagnostic value. This includes such disease entities as aspergillosis, Farmer's lung, and pigeon breeder's lung. More recently the fungus alternaria has been implicated in hypersensitivity lung disease. There is also some evidence that precipitating antibody detection is of value in diseases, such as systemic candidiasis, histoplasmosis, and coccidioidomycosis. Serum samples from patients seen at WRAMC who have diseases of the categories listed above or closely related clinical entities will be used. Sera will be processed by both standard double diffusion (Ouchterlony) technique and by counterimmunolectrophoresis method.

Progress and Results: Techniques for double immunodiffusion and counterimmunolectrophoresis of various precipitating antigen antibody reactions have been refined in the Allergy-Clinical Immunology Laboratory. In the past year 1,149 sera have been processed. Four hundred and thirty-one sera have been evaluated by double diffusion technique, 718 sera by the counterimmunolectrophoresis method. Antigens tested included several species of aspergillosis, candida albicans, micropolyspora faeni, thermoactinomycetes vulgaris, alternaria pigeon sera and pigeon droppings and baker's yeast.

Conclusions: The counterimmunolectrophoresis is the faster and generally more sensitive assay for the detection of precipitating antibodies to the abovementioned antigens. The presence of these precipitating antibodies has proven to be helpful in clinical evaluation of patients with respiratory disease, especially if this disease is of a hypersensitivity nature.

Publications: Symposium of Allergy and Pulmonary Diseases,  
Fitzsimons Hospital, 1974 and, CHEST, Vol 63, no 4, April  
1974, p 495-515.

Type of Report: Final

Work Unit No.: 3122

Title of Project: Comparison of Albuterol and Isoproterenol  
Aerosols in the Control of Bronchospasm  
Associated with Bronchial Asthma.

Principal Investigators: Major Blair Thrush, MC  
Charlotte Casterline, MD

Associate Investigator: Colonel George W. Ward, Jr., MC

Objectives: To compare the efficacy, safety and acceptability of albuterol aerosols with that of isoproterenol aerosols in relieving bronchospasm in patients with bronchial asthma.

Progress and Results: Fifteen patients completed the eight week double-blind single crossover comparison of aerosolized albuterol and isoproterenol. The data has been analyzed and the completed study is in the final process of submission to the Journal of Allergy and Clinical Immunology. Both aerosols were found to be equally potent bronchodilators at 15 and 30 minutes post inhalation. However, albuterol had statistically significant longer duration of action at 1, 1-1/2, 2 and 4 hours. These differences were noted in all three pulmonary functions monitored -- the FVC, FEV<sub>1</sub> and MMEF.

Thirteen of 15 patients found regular use of beta adrenergic aerosols useful and of these 13, ten preferred albuterol. Side effects were minimal from both drugs with four patients complaining of tremor following isoproterenol and one of nausea after albuterol.

Conclusions: As compared with aerosolized isoproterenol, aerosolized albuterol is an equally effective, longer acting bronchodilator with better patient acceptance and with fewer side effects.

Funds utilized FY-76: None

Funding requirements FY-77: None

Publications FY-76:

1. Proceedings of the 4th Annual Meeting of the Association of Military Allergists, Sept 10-11, 1975, Fitzsimons Army Medical Center, pages 28-37 (preliminary report).

2. Study submitted to the Journal of Allergy and Clinical Immunology.

3. Presented to the Southwest Allergy Society, May 1976.

Type of Report: Completed.

WORK UNIT NO. #3123

TITLE OF PROJECT: Study Immune Mechanisms in Systemic Lupus Erythematosus

Principal Investigators: Oliver J. Lawless, MD, LTC

Bernard Berne, PhD, MD

Objective: To simultaneously assess the function of the phagocytic T & B components of the immune system in the production of the symptoms of acute Lupus, and to compare these findings with those found during inactivity or remission of the disease.

Technical Approach: Lupus is an antigen induced disease, the major responsible antigen being double stranded DNA. The modulation of phagocytic, T & B cell function by antigen, antibody, and antigen antibody complex has pertinence not only to Lupus but to all infectious diseases where antigen persistence plays a role, but also to the mechanisms of transplantation, and tumour rejection. Lupus patients are unique in that the DNA antigen, and antibody systems can be isolated, purified, and tested in an vitro system on the cells of the patient without potential injury to him. Identification of the modulation mechanism in Lupus would have direct importance to host resistance to infection, and to transplant and tumour rejection, all of which are major thrusts of military research.

Inflammation in Systemic Lupus is caused by immune complexes. Patients with acute disease frequently have local deposition of complexes, high levels of free DNA, anti DNA, and elevated anti DNA levels after treatment of serum with anti DNase. They also have elevated levels of proteins and immunoglobulins, reduced levels of complement C3, C<sup>4</sup>, and CH50 and depressed T cell function. Rheumatoid factors are frequently found also. It is our hypothesis that complexes are deposited because of ineffective clearance by the phagocytic system. The mechanism for clearance of these complexes has not been directly defined in Systemic Lupus Erythematosus. It is our proposal to study the role of DNA ag, anti DNA antibody, and DNA-anti DNA complexes and rheumatoid factor on phagocytic, T & B cell function in acute active, and inactive Systemic Lupus Erythematosus (SLE). Immune complexes incite an inflammatory reaction at the site of their deposition eg. kidney, skin, lung, etc. Clearance of complexes from the circulation is by the RE system - monocytes and polys, of peripheral blood, and by fixed and wandering RE cells of parenchmal organs eg. liver, kidney, etc.

Phagocytic clearance has not been measured in SLE. All assays for functional activity of phagocytes depend upon indirect assays such as NBT test, iodine incorporation into protein and clearance studies using radio labelled macro-aggregated proteins. Clearance of DNA anti DNA complexes with radioactively labelled DNA would provide us with information more specifically relatable to phagocytic function in this disease.

T lymphocyte number and function have been shown to be reduced in acute untreated SLE. The mechanism responsible for this has not been defined. Three potential explanations for this phenomenon can be proposed (1) the presence of lymphocytotoxic factors, (2) the presence of blocking factors that are not cytotoxic, (3) the presence of immune complexes that "alter" lymphocyte reactivity, (4) the presence of antibody against T cell receptors in SLE sera. Fundamental to the understanding of blocking or enhancing factors on the T lymphocyte is definition of the role of complexes of DNA anti-DNA made up in antigen excess, equivalence and antibody and antibody excess, on these cells as we have shown that depending upon the Ag-Ab ratio of these complexes serum containing these complexes can be either enhancing or blocking to Ag and PHA induced T cell responses.

HLA profiles have been recently linked with a high statistical significance to certain diseases considered to be immunologically induced. HLA profiles in SLE have been reported to yield variable results. It is to be noted however that SLE serum can display blocking factor activity, in relation to lymphocyte function and HLA expression. Culture and washing of peripheral blood lymphocytes of SLE patients for twenty-four hours may yield additional HLA antigens not detectable at time zero. The relationship of this finding to the presence of DNA-anti DNA complexes needs further definition and confirmation. Antigen, and mitogen induced T cell responses result in lymphocytic blast transformation and lymphokine production in tissue culture. Lymphokines have shown to have specific effects on polys, and monocytes as well as other lymphocytes. If T cell responses are diminished by reason of cytotoxic factors or blocking factors then theoretically mediator release from T cells would be suppressed, and accordingly loss of T effect on poly and monocyte would be expected, a phenomenon that could contribute to decrease in phagocytic function and clearance of complexes.

The practical application of this project is as follows. If one can show that complexes present in serum are responsible for suppression of T cell directly and poly and monocyte indirectly then two potential treatment approaches are virtually free of toxicity. (1) Plasmapharesis for removal of complexes, (2) transfer factor therapy, made from the patient himself during the non-acute

man "suppressed" phase of this disease with a view to (a) removing the blocker, (b) boosting the deficient cell system responsible for defective clearance of complexes.

B2 microglobulin has been shown to be (1) produced by cells including T cells and monocytes in tissue culture, (2) to be metabolized by tubular epithelium cells of the kidney, (3) to be high in the serum of patients with severe renal disease, and (4) anephric patients, and to be (5) high in the urine of patients following transplantation. It has furthermore been shown to be similar to the C<sup>3</sup> domain on the H chain of IgG molecule and to be closely linked to the HLA antigen bound on the surface membrane of T lymphocytes.

It is theoretically possible therefore that serum levels of B<sub>2</sub>M will be altered in SLE, and urinary levels may be altered prior to evidence of clinical activity of the disease. Epstein has shown that free light chains in an effort to identify if these measurements afford earlier recognition of exacerbation of SLE. Within this overall project the following individual projects and protocols are proposed:

Phagocytic Function

- (1) Development of in vitro phagocytic assay using C<sup>14</sup> labelled DNA anti DNA complexes of fixed molar ratios and peripheral blood monocytes and polys,
- (2) Measurement of the effect of Ag (DNA) Ab (anti DNA) C<sup>1</sup>, and Rheumatoid Factor on this assay system,
- (3) Measurement of effect of lymphokines on this assay, from normal, and active and inactive SLE patients lymphocytes,
- (4) Measurement of effect of lymphotoxic serum on this assay.

Lymphocyte T Function

- (1) DH to battery of Ags,
- (2) Enumeration of T & B cell numbers by Rosette and surface staining technique,
- (3) Measurement of T cell response to Ag, MLC, PHA, PWM, DNA and DNA anti DNA complexes, in different molar ratios,
- (4) Measurement of the effect of Ag - Ab complexes in different molar ratios on the kinetics of T cell responses in normal and

SLE patients in response to Ag and PHA and PWM,

(5) Measurement HLA profile of SLE patients and the influence of time, Ag and Ab complex on this profile,

(6) Measurement of lymphokine production in SLE lymphocytes and the effect on macrophage migration inhibition and leukocyte migration inhibition.

B Cell Function

(1) Measurement of IgG, A, M, D and E levels,

(2) Measurement of antinuclear antibodies by FANA, and DNA binding technique,

(3) Measurement of C<sup>3</sup> and CH50 complement levels.

Immune Complexes (IC) Detection

Immune complexes will be detected by a solid phase sandwich radioassay using labelled Clq, a part of the first component of complement. Clq, a protein with a molecular weight of 600,000 can bind and precipitate with IC, although existing tests utilizing this reaction are generally either cumbersome or insensitive, they have been shown to be useful in documenting the rise in IC during exacerbations of SLE.

While several polyanions (DNA, Heparin, Endotoxin, etc.) are known to also bind to Clq, the specificity of the reaction with IC appears sufficient to enable this to form a sensitive assay for IC, as increased binding correlates with disease activity in SLE. Improvements in methodology such as the radioassay proposed here will allow an assessment of the actual effects of non-specific binding on the assay for IC.

Urine Proteins

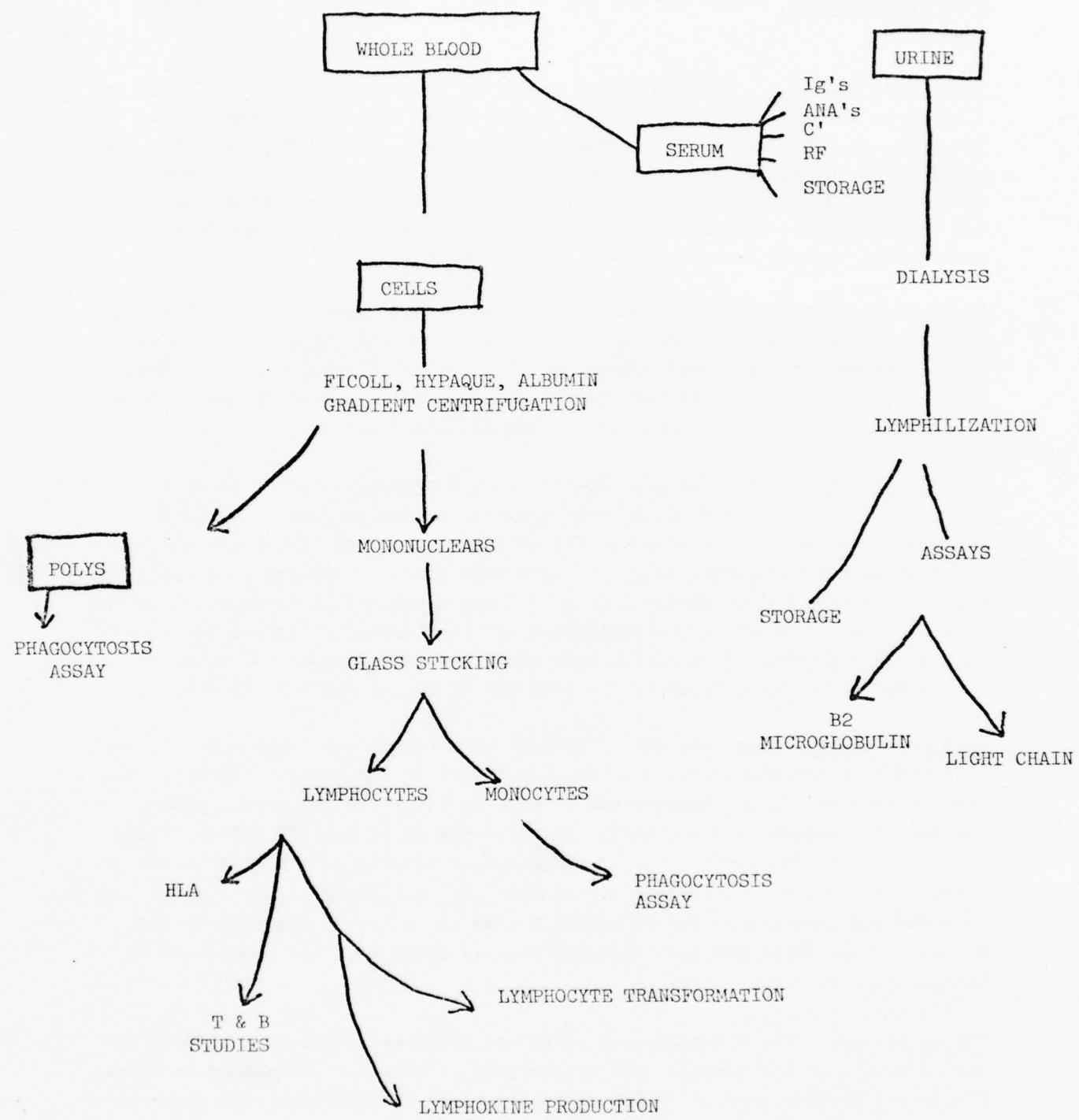
Measurement of urinary light chain excretion, and B<sub>2</sub> micro-globulin excretion in the urines of quiescent and active Lupus patients.

Plan: The techniques essential for completion of these studies are all currently in use in our laboratory having been developed during the past two years as follows:

- (1) Leukocyte, poly, monocyte, and lymphocyte separation.
- (2) Phagocytic function using  $C^{14}$  labelled immune complexes.
- (3) Lymphocyte transformation.
- (4) Lymphokine production and characterization.
- (5) Immunoglobulin levels.
- (6) T and B cell enumeration.
- (7) Antinuclear antibody measurement.
- (8) Immune complex detection.

Collaborative work will be carried out with Dr. Spees of Organ Transplant Service on HLA typing profiles and with Dr. R. Wistar of Navy Medical Research Laboratory for urine protein identification and quantitation of both light chain and  $B^2$  microglobulin excretion studies. All patients to be studied will be drawn from our clinic population of over one-hundred-fifty patients who fulfill American Rheumatism Association classification criteria for SLE. Activity of disease will be assessed upon the number of organs displaying signs of inflammation. After establishing the diagnosis and consent obtained a blood sample and twenty-hour collection of urine will be obtained.

The subsequent handling of blood and urine will be carried out as per diagram:



Progress and Results:

Phagocyte Function - This has not yet been set up and studied for logistic reasons.

Lymphocyte Function - Delayed hypersensitivity to a battery of 5 antigens, and lymphocyte transformation as measured by Eritiated thymidine incorporation into DNA, in response to medium alone or background; DNA, SKSD, PHA, Pokeweed mitogen PWM, as antigenic or mitogenic stimulants, were used. All above assays were run on 13 SLE patients, and 13 controls; in the presence of autologous and normal control plasma, before and after heat inactivation, and in the presence of varying doses of DNA as added antigen.

Background responses were slightly increased for SLE lymphocytes compared to controls. The mean value for SLE lymphocytes in normal plasma was 695, for normal lymphocytes in normal plasma  $342 = \text{Stimulation Index (SI)} = 2$ . The mean value of SLE lymphocytes in SLE plasma was 818 and for normal lymphocytes in SLE plasma  $832 = \text{SI} = 1$ , not significant. See Figures I and II.

DNA as Antigen - In preliminary experiments DNA from different sources KB cells, calf thymus, and E. Coli - was used in increasing doses from .1 to  $100 \mu\text{cg}$ , by addition to a standard PHA response of normal lymphocytes. It was found to be non-inhibitory unless at very high doses of  $100 \mu\text{cg}$  or greater and the only in some experiments. A dose of  $1 \mu\text{cg}$  was accordingly selected as the test dose for stimulation and inhibition although serial dilutions were used in some experiments. Stimulation over background was seen to DNA by both normal and lupus lymphocytes in the presence of normal and SLE plasma.

SKSD as Antigen - Mean response of control lymphocytes gave approximately 22,000 CPM in normal plasma, and slightly higher in SLE plasma. In contrast, the response of SLE lymphocytes was reduced to 8,000 CPM for normal plasma and further reduced in SLE plasma. The presence of excess DNA altered these responses on normal lymphocytes, causing slight reduction of CPM of normal lymphocytes in both control and SLE plasma. The responses of SLE lymphocytes to added DNA were unaffected in normal plasma and slightly enhanced by SLE plasma. Thus, added DNA gave variable results which were not consistent in the presence of SKSD.

PHA as Mitogen - The PHA responses of normal lymphocytes were not altered by use of normal or SLE plasma. SLE lymphocytes responses were reduced in normal plasma and further reduced in SLE plasma compared to controls. The presence of added DNA affected both normal and SLE lymphocytes by causing a reduction

in CPM, and this reduction was increased in the presence of SLE plasma over that of controls on both normal and SLE lymphocytes.

PWM as Mitogen - The response of control lymphocytes was slightly higher than SLE lymphocytes. Neither cell responses were appreciably affected by the presence of SLE or normal plasma. Added DNA caused reduction of CPM of normal lymphocytes to the approximate value found on SLE lymphocytes. See Figures I and II.

COMPARISON OF LYMPHOCYTE FUNCTION IN SLE PATIENTS WITH ACUTE VERSUS MILD OR  
INACTIVE DISEASE IN AUTOLOGOUS AND NORMAL PLASMA

TABLE I

PLASMA	SLE ACTIVE		CONTROL	
	NORMAL	SLE	NORMAL	SLE
Background	340	447	568	523
SKSD	10,597	2,917	13,947	2,671
PHA	17,723	9,942	45,793	11,743

PLASMA	INACTIVE SLE		CONTROL	
	NORMAL	SLE	NORMAL	SLE
Background	695	818	342	832
SKSD	8,327	7,562	20,269	27,101
PHA	45,793	40,870	55,320	55,239

Two patients with SLE had acute active disease. Eleven had mildly active or inactive disease. Both acute patients had significantly lower mean SKSD and PHA responses than the mean of the entire SLE group and controls. Furthermore, the responses to SKSD and PHA were further reduced by the presence of SLE plasma compared to control plasma. The sera of both of these active patients was checked and found to be negative for lymphocytotoxic antibodies against normal lymphocytes. Data on acute SLE cells and plasma is marked with asterisk in Figures I and II.

Effect of Plasma Fractions from Control, Inactive, and Active SLE Patients on Normal Lymphocyte Blastogenesis to SKSD and PHA.

The plasma of 1 control, 1 active, and 1 inactive SLE patient was fractionated by sephadex G200 column chromatography. Three main peaks were recovered corresponding to the IgM, IgG, and albumin peaks of normal human serum. The results of blastogenic responses to background and peak doses of SKSD, and PHA on normal human lymphocytes run in 15% normal human plasmas with  $40 \lambda$  of each fraction added are as in Table II and are reported as CPM thymidine incorporation into DNA.

TABLE II

	<u>CONTROL 1</u>	<u>ACTIVE SLE</u>	<u>INACTIVE SLE</u>
<u>Background</u>			
Whole			
Fraction I	570	299	300
II	441	293	523
III	436	280	1,442
<u>SKSD</u>			
Whole			
Fraction I	16,180	6,367	5,967 (3)
II	16,073	71 <sup>4</sup>	18,830 (0)
III	3,751	351	6,479 (1)
<u>PHA</u>			
Whole			
Fraction I	44,857	39,322	34,599
II	38,704	18,472	29,225
III	30,760	2,394	22,449

Summary of Results: These results suggest (1) lymphocytes of SLE patients have an intrinsic reduced responsiveness to SKSD, PHA, and PWM. (2) (Extrinsic) factors exist in SLE plasma which appear to cause further suppression of the reduced intrinsic lymphocyte blastogenic response to SKSD and PHA and in addition suppress lymphocyte blastogenesis in normal controls. (3) Clinically active SLE patients' plasma is greater than that of inactive SLE plasma in potentiating this intrinsic defect on SLE lymphocytes in response to SKSD and PHA, and it also reduces blastogenesis in normal control lymphocytes. (5) Added DNA in the dosage used appears to have a minimal suppressive effect on blastogenic responses of both normal and SLE lymphocytes, but this is greater in SLE plasma than in control plasma. (6) Fractions of control, active SLE, and inactive SLE plasma corresponding to IgM, IgG, and albumin fractions of normal serum sera demonstrated to have variable but significant suppressive effects on blastogenic responses to SKSD and PHA on normal lymphocytes, without alteration of background responses. The most potent suppressive in active SLE plasma was found in small molecular weight (40,000) fraction of acute SLE plasma through suppressive activity was detected in IgM and IgG. (7) Immune complexes made in variable molar ratios of Ag to Ab to produce Ag Excess, equivalence, and antibody excess, failed to enhance background stimulation of normal or SLE lymphocytes. (8) In one patient with acute active SLE found to have a potent plasma lymphocyte suppressing

factor and reduced intrinsic responsiveness treatment with high dose prednisone and cytoxan caused remission of the disease. When the lymphocytes were tested 3 months after initiation of treatment and while still on prednisone and cytoxan the intrinsic and extrinsic hypo-responsiveness had abated. Thus, the circulating plasma suppressing factor on lymphocyte function was more potent than an oral prednisone cytoxan regimen.

Conclusions: Intrinsic and extrinsic suppression of lymphocyte function has been defined in SLE lymphocytes compared with controls, and active SLE compared with inactive controlled or treated SLE. The nature of the intrinsic reduced responsiveness has not been defined. Extrinsic factors existent within IgM, IgG, and albumin fractions of acute SLE plasma impair blastogenesis of normal lymphocytes and thus are not auto-regulatory and non-specific in that they affect antigen induced blastogenesis as well as mitogenic responses. The number of patients in this study are small and need to be amplified especially in regard to active SLE.

B Cell Function - Immunoglobulin levels, FANA levels, DNA binding antibody items were measured on all 13 patients whose lymphocytes were studied as well as on several hundred other serum samples from SLE patients and the data is currently being analyzed.

Urine Proteins - Light chains and  $\text{B}_2$  microglobulin excretion in the urine has not yet been set up but this is planned in the near future.

Funds Utilized 1976: \$4,049, Funded \$13,921.

Funding Requested FY 77:

We request continued funding of this project as an essential component of the Rheumatology & Clinical Immunology fellowship training program as it integrates cellular and humoral immune mechanisms in the pathogenesis of disease. Our laboratory has not previously been involved in fractionation of serum proteins and preparation of purified antibody preparations. Further analysis of the role of antibodies and immune complexes and rheumatoid factors as well as small molecular weight serum factors pertinent to our current results requires setting up of the techniques of preparation and analytical electrophoresis, column chromatography, and polyacrylamide gel electrophoresis. The chief reason for under utilization of funds in 1976 was due to increased clinical and teaching responsibilities of staff. This year with an increased TDA to provide 4 staff, including a PhD, emphasis on research will be doubled.

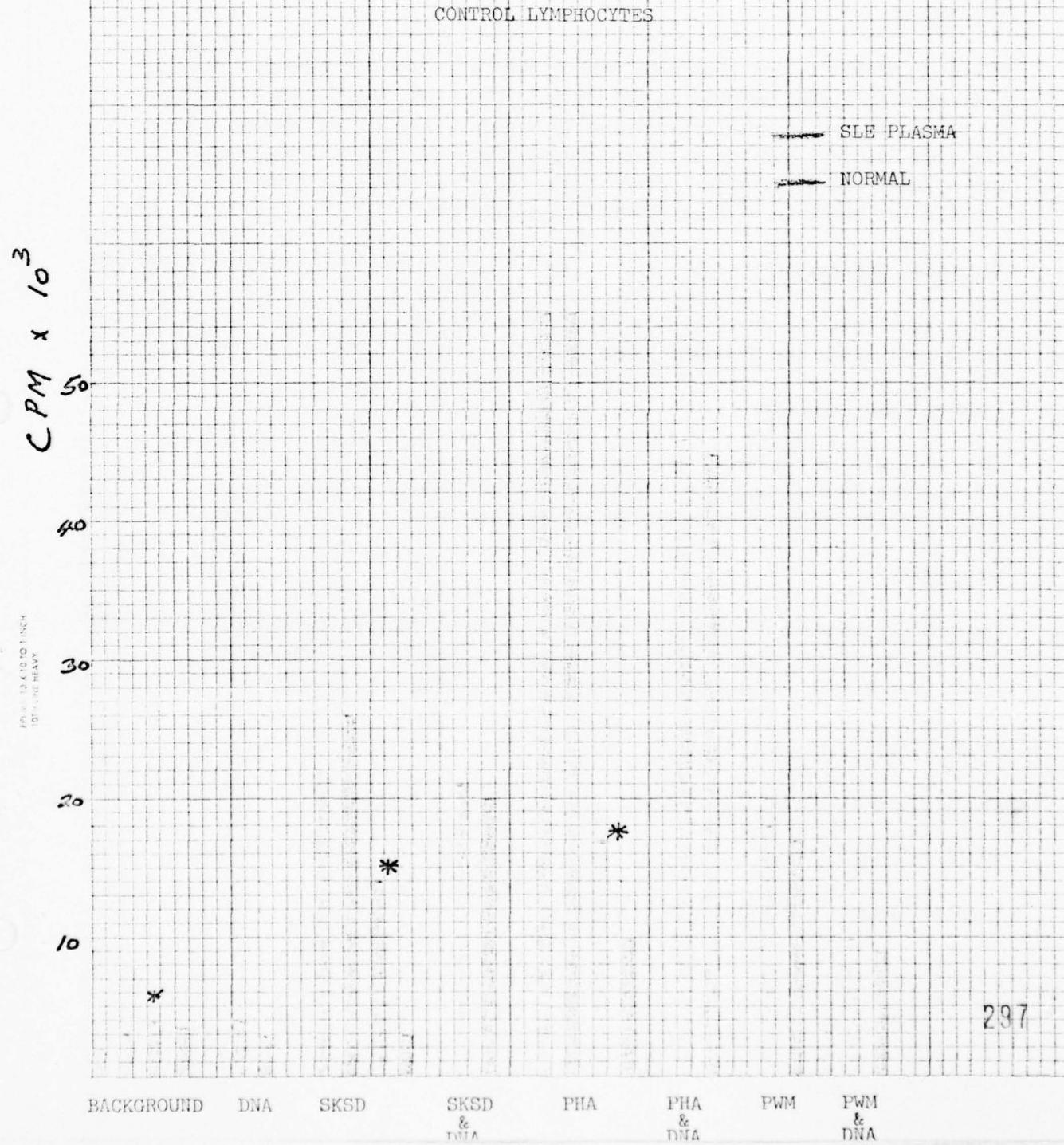
Personnel:	One GS 9, Step 8 Civilian Technician	3/5 Time at \$16,625 per year	\$ 11,196
Equipment:	Plasma Freezer - 35°	950.00	
	Conductivity Bridge	385.00	
	Conductivity Cell	55.00	
	Polyacrylamide Gel- Electrophoresis Equipment	1,500.00	
	UV Monitor for fraction, collector and recorder for UV monitor	1,975.00	
	Volumeter model 400 for fraction collector	325.00	
	Metering Pump, model 312	645.00	
	Columns for Sephadex chromatography	<u>173.00</u>	
	TOTAL	\$ 6,008.00	
Supplies:	Cellular Immune Testing		
	Radioisotopes	1,500.00	
	Media	232.50	
	Scintillation fluids	205.00	
	Scintillation Vials	4,000.00	
	Glassware and disposable wares	<u>2,062.00</u>	
	TOTAL	\$ 7,999.50	
	Humoral Testing		
	Radioisotopes	2,000.00	
	Chemicals	2,500.00	
	Anti-sera	2,000.00	
	Glassware	<u>1,500.00</u>	
	TOTAL	\$ 8,000.00	
Equipment Rental:			
	Water deionizer, 200	\$ 200.00	
Summary Sheet:			
	Personnel	\$11,196.00	
	Equipment	6,008.00	
	Supplies	15,999.50	
	Rental Equipment	<u>200.00</u>	
	TOTAL	\$33,403.50	

Publications: None

Type of Report: Interim

FIGURE I.

Mean values of the lymphocyte responses measured in CPM of 13 controls showing the effect of (a) autologous, (b) SLE-plasma, and (c) DNA on background, SKSD, and PWM responses. The second set of blocks marked with an asterisk \* represent the mean values for active SLE and their controls.



Work Unit No.: 3125

Title of Project: Histocompatibility antigens in patients with Anterior Uveitis Spondyloarthropathies.

Investigator:

Principal Investigator: LTC Oliver J. Lawless, MD, MC

Objective:

Histocompatibility antigens have been linked with various disease processes including Ankylosing Spondylitis, Reiter's Disease, and Psoriatic Arthritis when accompanied by Spondylitis. In all these entities Anterior Uveitis may occur suggesting that the host response may be modified by genetic factors. A study of patients with Anterior Uveitis for presence of HL-A 27 will be undertaken.

Technical Approach:

A HLA panel will be performed on all patients by the transplant laboratory using the Terasaki Technique.

Progress and Results:

This project was commenced and tests have been performed for us by COL Everett Spees Transplant Laboratory. The numbers to date are small and inadequate for statistical analysis.

Conclusions:

The results to date despite low numbers, however, conform to those reported by others that HLA B27 is present in greater than 90% of those tested with Ankylosing Spondylitis and Reiter's Syndrome.

Funds Utilized FY 76: None.

Funding Requested, FY 77: None

Publications: None.

Type of report: Interim

Work Unit No.: 3126

Title of Project: A Study of Mechanism Causing Polymyositis

Investigator:

Principal: Oliver J. Lawless, MD, LTC, MC

Objectives: (1) To evaluate the evidence for a delayed hypersensitivity mechanism in causing Polymyositis.

(2) To measure the incidence and titer of antibodies to Toxoplasma gondii in Polymyositis.

Progress and Results: This project failed to get off the ground for several reasons. The principal reason, however, was that inadequate numbers of fresh or untreated cases of Polymyositis were seen in the past year by our Service. The majority of patients seen by our Service were already on significant doses of corticosteroid or other potentially immunosuppressive medication.

Alternately patients referred were frequently symptomatic enough to warrant treatment with one or other of these agents, so that commencement of therapy could not be postponed prior to obtaining lymphocyte data. The project has therefore been abandoned at this time.

Conclusions: N/A

Funds Utilized FY-76: None

Funding Requested FY-77: None

Publications (FY 76): None

Type of Report: Terminated

Work Unit No.: 3130

Title of Project: The Effect of Viral Antigens on Lymphocyte Transformation in Multiple Sclerosis Patients

Investigators:

Principal: Major Charlotte L. Casterline, MC

Progress to Date: This study has met with considerable technical difficulty, especially in regard to lack of reproducibility of results, even in the normal control population. Serious questions have arisen as to the validity of performing transformation studies with viral antigens, due to the impure nature of these substances. Without considerable technical support from a viral immunologist this study cannot proceed. Since these resources are not available at this institution, it is recommended that this protocol be terminated. Should such resources be available in the future, this protocol could be restudied. The question of lymphocyte response in multiple sclerosis remains enigmatic and perhaps holds a clue to the nature of this disease.

Type of Report: Termination

Work Unit No.: 3132

Title of Project: Multicentric Study by the American Academy of Allergy on the Safety and Efficacy of Albuterol Tablets When Administered Chronically in the Treatment of Reversible Obstructive Airway Disease: Compared to Ephedrine.

Investigators:

Principal: Richard Evans, COL MC

Associates: George W. Ward, COL MC  
L. Blair Thrush, MAJ MC

Objectives: (1) To compare the efficacy of chronic administration of albuterol tablets and ephedrine in providing relief from bronchospasm occurring in chronic reversible obstructive airway disease.

(2) To compare albuterol and ephedrine regarding incidence, time of onset, and degree of tachyphylaxis.

(3) To compare albuterol and ephedrine with respect to duration of acute bronchodilatory effect.

(4) To compare albuterol and ephedrine regarding effects on heart rate and the nervous system.

Technical Approach: The study is in two parts, the first being a three month double-blind study. After a two week control period the patients are randomized to either ephedrine or one of two different doses of albuterol. The patients are subsequently seen at two week intervals for the next three months at which time daily symptom diaries including daily peak flow rates are examined. The effect of their double blind medicine on FEV<sub>1</sub> is measured serially for six hours. After a patient finishes the double blind period he enters the second 12 month phase of the study which involves taking albuterol on a regular basis in association with asthma medications as needed. Both during the 3 month double-blind phase and in the 12 month phase careful evaluation of the patients asthmatic status is made. In addition careful observation of laboratory, EKG X-ray and ophthalmologic parameters are made.

**Progress and Results:** Both phase I (the 3 month double-blind comparison phase) and phase II (the 12 month chronic administration of albuterol) have been completed. Twenty-seven patients finished phase I with 17 continuing and finishing phase II.

The code on phase I revealed which drug each patient had been on, either albuterol 4 to 6 mgm or ephedrine 25 mgm. After one month on 6 mgm of albuterol the patients were asked to compare the efficacy of 6 mgm of salbutamol with their recently completed double-blind medication. Of the nine patients who had previously been on ephedrine 25 mgm, five preferred 6 mgm of salbutamol, three felt there was no difference, and one preferred the ephedrine. Of the seven patients on 4 mgm of albuterol, four preferred 6 mgm of albuterol, two noted no difference and one felt that 6 mgm was less effective than 4 mgm. Of the nine patients who were continued on 6 mgm of albuterol from the double-blind to the open chronic study, six noted no change and three felt they were better. These patient preferences suggest but do not statistically prove that albuterol is a more effective bronchodilator than ephedrine. Also, there is a suggestion that 6 mgm of albuterol is more effective than 4 mgm. In retrospect, the doses of 4 and 6 mgm of albuterol chosen for the phase I comparison with 25 mgm of ephedrine were too low making both ephedrine and salbutamol equally effective (or ineffective). When the patients finally were placed on 8 mgm of albuterol for the last 11 months of the chronic study the bronchodilation was much more obvious.

Analysis of serial pulmonary functions after albuterol 4 or 6 mgm in general showed no significant change in FVC<sub>1</sub>, FEV<sub>1</sub> or MMEF. Occasional observations showed significant bronchodilation (i.e., a 20% increase of FEV<sub>1</sub> and this occurred more frequently following 8 mgm of albuterol. Ephedrine 25 mgm was also generally ineffective. Our patient population was weighted toward severe disease. These severe asthmatic patients, when taken off their regular bronchodilators for 6-12 hours prior to the study day, often arrived with deteriorating pulmonary functions. A single, particularly oral medication of any type is unlikely to provide significant relief particularly if given in suboptimal doses.

Side effects from both albuterol and ephedrine were minimal and consisted primarily of tremor which generally was mild and improved after taking either drug for one week. No laboratory, X-ray or ophthalmologic abnormalities were noted

either during phase I or phase II. Neither albuterol or ephedrine in the doses given had a significant effect on heart rate or blood pressure.

Because of the relative lack of effect of both ephedrine and albuterol it is difficult to evaluate tachyphylaxis. In the ten patients who consistently responded to albuterol there was no evidence of tachyphylaxis.

**Conclusions:** Salbutamol in a dose of 8 mgm orally is a mildly effective bronchodilator in severe chronic asthmatic patients. It is more effective in mild disease. Doses of 6 and 4 mgm of albuterol as well as 25 mgm of ephedrine are relatively ineffective in this population. No significant adverse clinical or laboratory effects of albuterol were noted during the total 15 months of study.

**Funds utilized FY-76:** None

**Funds required FY-77:** None

**Publications:** Because this study was part of a multicentric study sponsored by the American Academy of Allergy, no individual reporting is allowed. A combined report written by the head of the study will be published when all centers have completed their studies and the combined data analyzed.

**Type of Report:** Completed

Work Unit No.: 3133

Title of Project: Reconstitution of T-Cell/Delayed Hypersensitivity Immunity with Thymus Tissue

Investigators:

Principal: Arnold Levinson, M.D. MAJ MC

Associate: Richard Evans III, M.D. LTC MC

Objective: The purpose of this protocol is to have the capacity to reconstitute cellular immune function with thymic tissue. Individuals who require this form of therapy will be identified by protocol 3117.

Technical Approach: This has been outlined in Annual Report FY-75

Progress and Results: The patient with chronic mucocutaneous candidiasis described in Annual Report FY-75 underwent a second thymus transplantation following clinical relapse. Two weeks postoperatively, delayed skin reactivity was noted to candida for the first time. This was attended by resolution of candida skin lesions. Other in vitro responses, both specific and nonspecific, normalized. Throughout this entire time period, the patient was maintained on transfer factor as well.

Conclusions: The results of therapy in this patient suggested the potential utility of combined transfer factor, thymus transplantation in certain immune deficiency disorders.

Funds Utilized, FY-76: \$3,000

Time of Report: Termination

Date Prepared: 26 May 1976

Work Unit No.: 3134

Title of Project: The Role of Phospholipase A (PLA)  
Fraction of Honey Bee Venom in Hypersensitivity Reactions in Humans Allergic to Bee Sting.

Investigators:

Principal: Richard Evans, COL MC  
Associate: Harold Baer, MD

Objectives: The objective of this protocol is to determine if hypersensitivity reactions to the phospholipase A fraction of honey bee venom can be demonstrated in humans allergic to the bee sting. In addition, correlations of specific IgE antibody leukocyte histamine release in these patients will be determined.

Technical Approach: Patients presenting to the Allergy Clinic at Walter Reed Army Medical Center with a history of sensitivity to insect sting were entered into this study. The phospholipase A for this study was approved by Federal Food and Drug Administration IND for a skin testing. In addition, the patients were skin tested with standard bee venom extract and whole body extract available in the Allergy Clinic. The skin test titration technique was used for the skin tests. In addition, in vitro leukocyte sensitivity to these antigens for histamine release and serum RAST (specific IgE) titers were performed.

Progress and Results: Fifteen patients presenting to the WRAMC Allergy Clinic with a complaint of having had a reaction to a bee sting were selected for study. Careful history revealed that these patients constituted three groups:

1. Patients with systemic reaction to the sting of a honey bee.
2. Patients with systemic reaction to the sting of a vespid.
3. Local reactions to either of the above.

The patients who had sustained systemic allergic reactions to a sting of a honey bee manifest skin reactivity to honey bee venom in all cases in concentrations of 10 ng/ml or less. The skin sensitivity to the whole body of the insect was quite variable. Skin sensitivity with the phospholipase A fraction by endpoint titration was also variable. The response ranged from 10 ng/ml to 10,000 ng/ml.

In this group of patients with systemic reactions to a honey bee sting whose skin test response to one ng/ml or 10 ng/ml of honey bee venom appears to be indicative of immediate hypersensitivity. The presence of IgE antibody activity was confirmed in vitro by either honey bee venom induced leukocyte histamine release or RAST titers of greater than 1.4 or both in all but one of the patients.

Sensitivity to honey bee venom or to phospholipase A fraction was not found in the group of large local reactions or the group of reactions to the sting of a non honey bee insect, a vespid.

Among these 15 patients who presented to our clinic, there were eight patients who were sensitive to honey bee venom and seven patients who were not. Including the in vivo and in vitro measurements, 59 tests were performed. The skin test titration procedure with honey bee venom was the most reliable in identifying the honey bee sensitive patient. The RAST titer to honey bee venom was correct in 87% of the patients tested and the leukocyte histamine release correlated with patient's history in 79% of the patients tested. The test using the phospholipase A fraction was less reliable. Apparently phospholipase A was not consistently the major allergen in the honey bee sensitive patients of this study population.

**Conclusions:** In the evaluation of a patient with a history of systemic reaction to a stinging insect, skin testing with honey bee venom is the best diagnostic agent. Skin testing with a whole body extract consistently failed to identify the truly sensitive patients. The phospholipase A fraction of the honey bee venom also failed to identify the honey bee venom allergic patient. Apparently this is a consequence of patients being sensitive to a component of a honey bee venom other than phospholipase A.

**Publications:** This paper was presented to the American Academy of Allergy in March 1976.

1) Harold Baer, Richard Evans and Michael L. Hooton, The Multiple Allergens of Honeybee Venom. J Allergy Clin Immunol 57:210, (Abs 51) Mar 1976.

2) Richard Evans, Harold Baer, C. V. Battista and G. W. Ward, Jr., Clinical Significance of Skin Testing with Phospholipase A Fraction of Honeybee Venom in Patients Sensitive to Insect Stings. J Allergy Clin Immunol 57:211, (Abs 53) Mar 1976.

Type of Report: Final.

Work Unit No.: 3135

Title of Project: Determination of the Reproducibility and Reliability of the Methacholine Bronchial Challenge Test in the Diagnosis and Prognosis of Asthma.

Principal Investigator: George W. Ward, Jr., COL MC

Objectives: To evaluate the usefulness of the methacholine bronchial challenge technique in the characterization of the clinical disease, asthma.

Technical Approach: By means of the bronchial challenge technique as defined by the Bronchial Challenge Committee of the clinical research centers of the Institute of Allergy and Infectious Disease, NIH, patients with asthma have been studied using a dosimeter which measures doses of methacholine and following which regular spirometry pulmonary performance measurements are made.

Progress and Results: Ninety-four patients with asthma and/or allergic rhinitis have been studied by means of the methacholine technique. We find the methacholine challenge to be capable of eliciting bronchial spasms measurable by changes in spirometry in patients with asthma and in certain patients with allergic rhinitis. Normal patients without bronchial airway disease do not respond to methacholine in this fashion. The technique has been shown to be quite repeatable in given patients repeated at different dates.

Conclusions: The methacholine challenge technique has now become an accepted means of characterizing asthma and has now become a part of our clinical evaluation of many asthmatic patients at this Medical Center and at other medical centers within the country. The technique has moved the realm of clinical research to clinical diagnosis.

Publications:

1. J of Allergy-Clin Immunol (Abstract) Feb 75.
2. Paper also presented to the Southwest Allergy Soc meeting, San Antonio, Texas, May 1976.

Type of Report: Final

Work Unit No.: 4100

Title of Project: Evaluation of the Vabra Aspirator in Obtaining Diagnostic Biopsies of the Endometrium.

Investigators:

Principal: Donald Walters, M.D.

Associates: Robert C. Park, COL, MC and Warren E. Patow, COL, MC

Objectives: The objective to this research are to determine whether an outpatient suction curettage of the endometrial cavity is as effective as a D&C of the endometrial cavity done under general anesthesia in the hospital.

Technical Approach: A small suction curette in to the endometrial cavity attached to suction and the endometrium removed. The patient then undergoes a D&C for abnormality.

Progress & Results: The study has been successfully completed. Three hundred patients underwent an outpatient Vabra Aspiration curettage and then within 24 hours had a conventional in hospital D&C under general anesthesia. Eighty-nine percent of the patients had successful vabra aspirator curettage and 95% of those completed were correctly diagnosed by aspiration. There were no uterine perforations or other major complications during the study.

Conclusions: The outpatient curettage was determined to be safe, reliable, economic and effective as a diagnostic tool. Patient acceptance was very good.

The paper has been accepted for publications in Obstetrics & Gynecology. The title, "Diagnostic Outpatient Aspiration Curettage." by Donald Walters, M.D., David Robinson, COL, MC Robert Park, COL, MC and Warren E. Patow, COL, MC

Funding Requirements:

Personnel: Christine Galope, OB-GYN Clinical Research Secretary, GS-5, is essential to maintain outpatient charts on these patients and to maintain inpatient charts to correlate the effectiveness of outpatient and inpatient procedures.

Type of Report: Completed.

Work Unit No.: 4102

Title of Project: Study of the Relationship Between Growth Pattern and Lymphangetic and Vascular Involvement in Women with Stage IA Cervical Cancer.

Investigator:

Principal: Robert C. Park, COL, MC

Associate: Samuel Goodloe, LTC, MC

Objectives: The objective of this study is to determine the motive spread of squamous cell carcinoma of the cervix.

Technical Approach: Patients are receiving surgery and histologic examination of tissues removed in a defined manner in order to gain maximum information from studying of the growth of this tumor.

Progress & Results: 27 patients have been entered in this study from Walter Reed and 337 from the entire G.O.G. To date there are no statistical results reported, however, there have been no positive lymph nodes.

Conclusions: None to date.

Funding Requirements: No local funds are necessary as this is a Gynecologic-Oncology Group funded protocol.

Type of Report: Interim

Work Unit No.: 4103

Title of Project: Treatment of Women with Disseminated or Recurrent Advanced Ovarian Cancer with Alkeran Alone or in Combination with 5-Flurouracil or in Combination with 5-Flurouracil and Actinomycin D.

Investigators:

Principal: Robert C. Park, COL, MC

Associates: Warren E. Patow, COL, MC Donald A. Simsen, COL, MC and Johannes Blom, M.D.

Objectives: The objective is to determine whether cancer of the ovary is better treated by single treatments regimens double, or triple treatment regimens of chemotherapy.

Technical Approach: Patients are being randomized into different treatment protocols and length of survival and progression free intervals are studied.

Progress & Results: 13 patients are entered in this study from WRAMC and 472 from the entire G.O.G. Single agent alkalyting therapy is as efficacious as other combinations.

Conclusions: Single agent chemotherapy is as efficacious as other combinations of chemotherapy for treatment of advanced ovarian carcinoma, and is less toxic. A GOG publication will be forthcoming.

Funding Requirements: No local funds require inasmuch as this protocol is being funded through the Gynecologic-Oncology Group.

Type of Report: Completed.

Work Unit No.: 4104

Title of Project: Post-Operative Treatment of Women with Stage III  
Ovarian Cancer by Radiotherapy or Chemotherapy  
Either Alone or in Both Sequences.

Investigators:

Principal: Robert C. Park, COL, MC

Associate: Warren E. Patow, COL, MC, Donald A. Simsen COL, MC,  
Thomas Stoffel, MAJ, MC and Johannes Blom, M.D.

Objectives: The objective of this study is to determine whether  
radiotherapy or chemotherapy is better in treating  
patients with advanced carcinoma of the ovary.

Technical Approach: Patients will be randomized between radiotherapy  
or Alkeran either alone or in sequence of one  
following the other.

Progress & Results: Ten patients are entered in this study from WRAMC  
and 313 from the entire G.O.G. To date there is  
no statistical difference in treatment regimens  
although a trend is showing radiation therapy  
alone to be less effective in the sub optimal  
group (residual tumor masses greater than 3cm).

Conclusions: None to date.

Funding Requirements: No local funds are required since this is  
funded through the Gynecologic-Oncology Group.

Type of Report: Interim

Work Unit No.: 4105

Title of Project: Treatment of Women with Advanced Cervical Cancer  
Confined to the pelvis with hydroxyurea or Placebo  
Both in Combination with Radiation.

Investigators:

Principal: Robert C. Park, COL, MC

Associate: Warren E. Patow, COL, MC, and Donald A. Simsen, COL, MC  
Thomas Stoffel, MAJ, MC

Objectives: The objectives of this study are to determine whether hydroxyurea adds to therapeutic benefit of radiotherapy.

Technical Approach: Patients are randomly either given or not given hydroxyurea with radiotherapy with advanced cervical cancer.

Progress & Results: The data on 331 patients would seem to show radiation and hydroxyurea to be the superior treatment arm.

Conclusions: A G.O.G. publication is forthcoming.

Funding Requirements: No local funds are required because this is funded through the Gynecologic-Oncology Group.

Type of Report: Completed.

Work Unit No.: 4106

Title of Project: Post-Operative Treatment of Women with Resectable Ovarian Cancer with Radiotherapy Alkeran or No Further Treatment.

Investigators:

Principal: Robert C. Park, COL, MC, Warren E. Patow, COL, MC, Thomas Stoffel, MAJ, MC and Johannes Blom, M.D.

Objectives: To determine the best approach to possible treatment of patients with Stage IA and IB ovarian cancer.

Technical Approach: Post-operative patients with ovarian cancer Stage IA and IB which is totally removed will be treated with either radiotherapy, chemotherapy or no further treatment.

Progress & Results: Thirteen patients have been entered from Walter Reed and 126 from the entire G.O.G. To date there are no differences in the three treatment arms.

Conclusions: None to date.

Funding Requirements: No local funds are required in this protocol because it is funded through the Gynecologic-Oncology Group.

Type of Report: Interim

Work Unit No.: 4107

Title of Project: Clinical Evaluation of the Female Urethra and  
Urethrovesical Function in Patients with Urinary  
Stress Incontinence.

Investigators:

Principal: Michael K. Kowalski, MAJ, MC

Associate: George E. DeShan

Objectives: The objectives are to determine abnormal urethral internal anatomy and its relationship to stress incontinence by comparing air urethroscopy with the conventional bead chain cystourethrogram. Normal patients will be used in order to determine what constitutes normal urethrovesical anatomy. This will be done on patients undergoing cystoscopy for other reasons.

Progress & Results: Thirty-six patients have been entered into this study as of this date. Anticipated completion is Spring 1977. A regular scheduled urethroscopy/stress incontinence clinic has been established with Department of Obstetrics and Gynecology.

Conclusions: None to date.

Funding Requirements:

Personnel: Christine Galope, OB-GYN Clinical Research Secretary, GS-5, will be essential for accumulation of tabulation of results and maintaining patient follow-up and communication with patients. No other funding requirements.

Type of Report: Interim

Work Unit No.: 4112

Title of Project: Diagnosis and Pretreatment Evaluation of Benign and Malignant Uterine Abnormalities Using the Operating Hysteroscope.

Investigators:

Principal: Donald A. Simsen, COL, MC

Associate: Robert C. Park, COL, MC and Warren E. Patow, COL, MC

Objectives: To determine the usefulness or lack of same of the hysteroscope in detecting uterine abnormalities.

Technical Approach: A hysteroscope utilizing dextran solution to the uterine cavity will be used to visualize the inside of the uterus.

Progress & Results: Awaiting the arrival of 32% dextran, 70 which has not been released by FDA. We will soon have some for use in hysterectomy specimens.

Conclusions: None to date.

Funding Requirements:

Personnel: Christine Galope, OB-GYN Clinical Research Secretary, GS-5, will be essential for maintaining files of these patients which will contain findings at the time of hysteroscopy plus findings at the time of any further procedures such as hysterectomy, and correlating the two. No other funding requirements.

Publications: None

Type of Report: Interim

Work Unit No.: 4113

Title of Project: Cooperative Gynecologic-Oncology Group

Investigators:

Principal: Robert C. Park, COL, MC

Associate: Warren E. Patow, COL, MC and Donald A. Simsen, COL, MC,  
Samuel Goodloe, LTC, MC, Thomas Stoffel, MAJ, MC and  
Johannes Blom, M.D.

Objectives: The Department of Obstetrics and Gynecology is involved  
with the nationally organized Gynecologic-Oncology Group  
which contains 32 of the major medical centers in the  
country who are interested in this area of gynecology  
treatment.

Progress & Results: WRAMC is active in 10 protocols involving treatment  
of ovarian carcinoma, cervical carcinoma, adeno-  
carcinoma of the endometrium and uterine sarcoma.  
To date 490 patients have been registered in this  
group from WRAMC and 97 patients have been placed  
in protocol studies.

Funding Requirements: No local funding is requested as this group is  
supported by the N.C.I. through the University of  
Buffalo.

Type of Report: Interim

Work Unit No.: 4116

Title of Project: The Evaluation of Fetal Systolic Time Intervals and Beat to Beat Interval Variations in Fetal Heart Rate as Early Indicators of Fetal Maturity and Fetal Distress.

Investigators:

Principal: Frank C. Miller, LTC, MC

Associate: John Read, MAJ, MC and Henry Klapholz, MAJ, MC

Objectives: The objectives are to determine when fetal heart rate electrical production patterns develop in the maturing fetus and how they might change as very early indications of fetal distress before labor commences.

Technical Approach: This will be done by external heart monitoring and doing fetal heart ultrasonography to intergrate these factors on a multiple channel recorder along with maternal heart beat and maternal blood pressure to evaluate the impact of these various factors.

Progress & Results: All equipment is now available and the project has begun. The addition to our OB staff of a Perinatal Research Nurse is expected to facilitate the completion of this study.

Conclusions: None to date.

Funding Requirements:

Personnel: Christine Galope, OB-GYN Clinical Research Secretary, GS-5, is needed for handeling the large volume of data collected and storage and retrieval. Her keeping is essential in this project.

Type of Report: Interim

Work Unit No.: 4118

Title of Project: Precancerous Lesions of the Cervix

Investigators:

Principal: Robert C. Park, COL, MC

Objectives: To determine the value of the colposcope and evaluating abnormalities of the uterine cervix.

Technical Approach: Patient with cervical pap smear abnormalities undergo colposcopy and biopsies of abnormal epithelium. They then undergo cold-knife conization. Correlations of these two tissue sampling techniques are made.

Progress & Results: An excellent correlation between colposcopic directed biopsies and cone biopsies has occurred. Actually one patient, however, did only have a dysplasia of the cervix on directed biopsy and did indeed have a microinvasive cancer of the cervix at the time of conversation. So further investigation of the use of this procedure is needed.

Conclusions: Paper in print by Spruce and Rogers, OB-GYN Journal.

Funding Requirements:

Personnel: Christine Galope, OB-GYN Clinical Research Secretary, GS-5, is needed to correlate histologic reports from cervical biopsies.

Type of Report: Completed.

Work Unit No.: 4122

Title of Project: The Management of Cervical Cancer, Stage IB

Investigators:

Principal: Robert C. Park, COL, MC

Associate: Warren E. Patow, COL, MC and Donald A. Simsen, COL, MC

Objectives: The objective of this research is to evaluate the use of radical surgery in treatment of Stage IB cancer of the cervix and to evaluate adjunctive diagnostic measures in relationship to the diagnosis of metastatic disease. Pedal lymphangiograms are being performed prior to radical surgery to evaluate their effectiveness and to determine the false negative rate of lymphangiograms.

Progress & Results: Continuing ongoing study, re-evaluation on a yearly basis.

Conclusions: To date the use of surgery and squamous cell carcinoma of the cervix appears promising. No conclusions can be seen to date as far as the evaluation of the lymphangiography.

Funding Requirements:

Personnel: Christine Galope, OB-GYN Clinical Research Secretary, GS-5, is required for maintenance of follow-up of the patients and for maintaining records and files of diagnostic and treatment modalities these patients have undergone.

Publications: Leman, MH, Park, RC, et al: Pretreatment Lymphangiography in Carcinoma of the Uterine Cervix.

Type of Report: Completed

Work Unit No.: 4124

Title of Project: Fetal Intensive Care Monitoring in a Long-Range Continuing Project.

Investigators:

Principal: Frank C. Miller, LTC, MC

Associate: Gary Broadnax, MAJ, MC and Norman Nechoes, MAJ, MC

Objectives: The objective of this research is to evaluate the usefulness of fetal monitoring and labor in detecting early fetal distress and abnormal fetal heart rate patterns. Beginning 1 July 1974 an increased effort was made to monitor all labor (where feasible) utilizing electronic clinical fetal monitoring equipment. A work sheet is completed on each patient and all the FHR tracing are being reviewed. To date the clinical correlations between normal FHR and good 1 & 5 minute Apgar scores has been excellent. Currently work is being done to develop and test a standard code sheet which may be utilized with a computer.

Conclusions: There are indications that several heart rate patterns in fetus during labor correlate with abnormal clinical situations, this is being evaluated in detail in an on going study.

Funding Requirements:

Personnel: Christine Galope, OB-GYN Clinical Research Secretary, GS-5, is needed to continue accumulation and evaluation of data. No other funding is needed.

Type of Report: Interim

Work Unit No.: 4126

Title of Project: The Clinical Evaluation of a Rapid Method for Presurgical Cleansing of the Hands.

Investigators:

Principal: Frank C. Miller, LTC, MC

Associates: Lawrence Decker, MAJ, MC, John Read, MAJ, MC  
Arthur Gross, COL, DC and Duane Cutright, COL, DC

Objectives: To compare the effectiveness of a 90 second pulsed jet hand and forearm wash with a standard 10 minute presurgical scrub.

Progress & Results: The evaluation of a new rapid method of pre-surgical cleansing has been done in several non-clinical studies at the Walter Reed Army Institute of Dental Research. The purpose of the paper is to compare the effectiveness of a 90 second jet wash method to the standard 10 minute presurgical scrub in a clinical setting.

Interns, Residents, and Staff of the Department of Obstetrics and Gynecology of Walter Reed Army Medical Center submitted finger tip cultures before and after 5-90 second jet washings and 5 traditional 10 minute scrubs. The results revealed a 10 minute scrub no more effective than the 90 second jet wash scrub. We feel the jet wash offers distinct advantage in the amount of time saved, the standardization of cleansing, and the decreased irritation.

Conclusions: The final results are being tabulated and the paper will be written within the next 6 weeks. In addition, a documentary film is being made of the procedure. This film has been accepted as part of a scientific exhibit for the Armed Forces Obstetric and Gynecology meeting in September 1976. The film was produced by the Department of Motion Picture Production Division of Medical Audio Visual Services with support from the Department of Dental Research at WRAIR.

Type of Report: Interim

Work Unit No.: 4127

Project Title: A Prospective Evaluation of Intrauterine Infection Associated with Fetal Monitoring.

Principal Investigator: Howard M. Listwa, CPT MC

Objective: To determine whether the appearance of polymorphonuclear leukocytes (PMN's) or bacteria could predict intrauterine infection.

Progress: Amniotic fluid samples from ninety-five internally monitored patients were examined. All patients delivered vaginally. More than one PMN per oil field were seen in 32% of patients, bacteria were seen in specimens from 52% of patients, organisms were grown in 93% of specimens. The overall maternal infection rate was only 6.3%. Infection developed in only 10% of patients with PMN's in the fluid and 6% of patients with positive grams stain or culture. For patients who deliver vaginally, the appearance of PMN's or bacteria does not predict infection.

Publications: The Predictability of Intrauterine Infection by Analysis of Amniotic Fluid. Howard M. Listwa, DO MAJ MC, Arthur S. Dobek, PhD, John Carpenter, MD MAJ MC, Ronald S. Gibbs, MD MAJ MC. Presented in part at AFD-ACOG, San Antonio, Texas, 6 Oct 75; and ICAAC, Washington, D.C., 25 September 1975.

Type of Report: Completed

Work Unit No: 4501

Title of Project: Clinical Evaluation of Fluorescence Scanning of the Thyroid with Americium 241 Source.

Investigators:

Principal: Merrill C. Johnson, M.D., Colonel, M.C.

Associate: Robert J. Corcoran, M.D., Major, M.C.

Objectives: To compare the clinical efficacy of fluorescence scanning with ultrasound scanning and with radionuclide scanning in evaluating patients with thyroid disease.

Technical Approach: A 300 square mm SI(L) detector mounted with a 20 Curie source on a Picker Magnascanner will be used to qualitatively and quantitatively evaluate stable thyroidal iodine in a group of 50 patients with various types of thyroid disease. These fluorescence studies will be compared to standard radionuclide scans and with ultrasound scans.

Progress & Results: Quantification of thyroidal iodine has been accomplished using a modified fluorescent scanning system of the concentric source-detector design. During the scan, the net counts due to K-alpha characteristic X-rays of iodine are determined with two sets of single channel analyzers (SCA's) and digital counters. The window of one SCA is centered at  $28.5 \pm 0.9$  keV. The window of the second SCA is centered at the same energy and adjusted so that in the absence of iodine the count rate is twice that of the first window over soft tissue. Net counts due to iodine are taken as twice the count in the first window minus the count in the second window. Calibration of net counts versus milligrams of iodine was done in phantoms. Accuracy of the technique was evaluated by comparing in vitro chemical and fluorescence analysis in 12 cadaver thyroid glands. The correlation was significant with a coefficient of 0.92 ( $P < .01$ ).

To date over 400 quantitative studies have been performed in 250 patients. A set of 30 normal subjects had an average thyroidal iodine content of  $10.1 \pm 3.9$  mgm. The results for several major diagnostic categories were: untreated Graves' disease, 28 patients,  $24.4 \pm 9.9$  mgm; diffuse euthyroid goiter, 12 patients,  $16.1 \pm 7.42$  mgm; primary hypothyroidism, 7 patients, 0.5 mgm, and nontoxic multinodular goiter, 23 patients,  $7.3 \pm 4.1$  mgm. Follow-up studies on patients treated for Graves' disease both medically and with I-131 revealed elevated iodine contents in 6 persistently hyperthyroid patients, lower than average normal amounts in 16 euthyroid patients and only trace amounts in four hypothyroid patients.

Thyroid gland iodine content is an important parameter of thyroid status. Accurate in vivo measurement may be accomplished with quantitative fluorescent scanning.

**Conclusions:** The fluorescent scanning device has proven simple to operate and maintain. It has provided excellent scans and quantitative information concerning the I-127 content of the thyroid gland. It has already provided clinical data of importance and will in the next year be the basis for several clinical investigations.

**Funding Requirements:** \$500.00

**Publications:** None to date.

Work Unit No: 4502

Title of Project: Plasma Angiotensin Levels and Response to Antihypertensive Therapy in Essential Hypertension.

Investigators:

Principal: Robert J. Corcoran, M.D., Major, M.C.

Associate: Jules Bedynek, M.D., Colonel, M.C.

Associate: Robert J. Kaminski, M.D., Major, M.C.

Objectives: To reinvestigate the effect of various antihypertensive medications as they may be related to angiotensin levels determined during the original evaluation of hypertensive patients.

Technical Approach: Use of a commercially available angiotensin kit to determine angiotensin levels in 100 consecutive volunteers who come to the cardiac clinic for evaluation of a hypertensive state. The patients to be placed on antihypertensive medications thought to be effective in "high renin" and "low renin" hypertension, their blood pressure response measured in this related to initial angiotensin levels.

Progress & Results: Prior to the current year no attempt was made to generate normal values by relating the 24-hour urine sodium to the plasma renin level. This was not found to be feasible in many clinical situations. Effort was made to evaluate the renin values among normals after furosemide stimulation as suggested by Wallach and Dawson. In this test the patient was given 60 mg of furosemide and five hours later a blood sample was taken. The distribution of renin values found among normal patients did not match that found by Wallach and Dawson. It was considered that this was due to generating the angiotensin I at a pH of 7.2 instead of the pH of 6.8 as suggested by Wallach and Dawson. In the coming year the same commercial kit used by Wallach and Dawson with their modifications, will be used. Fifty normal patients will be evaluated in an effort to reproduce the distribution of renin values as found by Wallach and Dawson.

Conclusions: None to date.

Funding Requirements:

FY 77 \$1,100.00 (supplies and antihypertensive medication)

Publications: None

WORK UNIT: 4513

TITLE OF PROJECT: Clinical Evaluation of Indium 111-Chloride

INVESTIGATORS: Principal: Merrill C. Johnson, COL, MC

Associate: Robert J. Corcoran, MAJ, MC

PROGRESS AND RESULTS: A total of six bone marrow studies were performed successfully. Interpretation of scans correlated with clinical findings, where bone marrow biopsies revealed inadequate tissue specimens. The addition of two high resolution cameras with dual scanning channels should increase the efficiency of this study and cause an increase in the number of studies requested.

CONCLUSIONS: Bone marrow scanning with Indium 111-Chloride can be a significant aid to the physician when other methods are inadequate.

FUNDING REQUIREMENTS: None

TYPE OF REPORT: Interim

WORK UNIT NO.: 4514

TITLE OF PROJECT: Clinical Evaluation of Indium 111-DTPA

INVESTIGATORS: Merrill C. Johnson, COL, MC

ASSOCIATE: Robert J. Corcoran, MAJ, MC

PROGRESS AND RESULTS: A total of 18 patients were studied during the report period. After review and correlation with clinical, pathological and radiographic findings, there were 12 patients with positive results, 4 negatives and 2 technically unsatisfactory.

There were no false positives and no false negatives. There were also no adverse reactions.

CONCLUSIONS: The advantages of this procedures are evident from the 100% positivity rate, however, the use of computerized axial tomography will supplant this methodology except where CSF leaks are suspected.

STATUS: On going.

FUNDING REQUIREMENTS: None.

Work Unit No: 4515

Title of Project: Broad Clinical Evaluation of  $^{99m}$ Technetium Labeled Stannous Glucoheptonate as a Diagnostic Agent for Studying the Kidney.

Investigators:

Principal: Merrill C. Johnson, COL MC

Objectives: To evaluate  $^{99m}$ Stannous Glucoheptonate as a diagnostic aid in studying the dynamics of brain and renal blood flow.

Technical Approach: See approved protocol.

Progress & Results: Four patients with chronic renal failure were studied using  $^{99m}$ Tc Glucoheptonate with adequate dynamic flow analysis. This diagnostic procedure is particularly well suited for studies of renal tubal absorption.

Conclusions: None

Funding requirements: N/A

Personnel: N/A

Equipment: N/A

Supplies: N/A

Travel: N/A

Publications: None

Work Unit No: 4516

Title of Project: Clinical Evaluation of  $^{123}\text{I}$ Iodine

Investigators:

Principal: Robert C. Corcoran, MAJ MC

Objective: Patients referred to Nuclear Medicine Service for evaluation of thyroid disorders will routinely be imaged following oral administration of  $^{123}\text{I}$ Iodine.

Technical Approach: The radionuclide  $^{123}\text{I}$ Iodine is metabolized in the same fashion as  $^{131}\text{I}$ Iodine. Administration will be orally followed by imaging of the thyroid gland.

Progress and Results: This radionuclide was not evaluated during the past year because of the report of Pinsley, et al demonstrating the superiority of  $^{99\text{m}}\text{Tc}$  pertechnetate in camera imaging of the thyroid with a pinhole collimator.

Conclusions: None to date, but studies with  $^{123}\text{I}$ Iodine may be indicated at a later date.

Funding Requirements: N/A

Personnel: N/A

Equipment: N/A

Supplies: N/A

Travel: N/A

Publications: N/A

Work Unit No: 4517

Title of Project: I-131 Induced Hypothyroidism: Relationship to Iodine Metabolism and Measurement of Onset by RIA - T4 Determination.

Investigators:

Principal: Merrill C. Johnson, Colonel, M.C.

Objective: To produce a clinically satisfactory method of calculating an I-131 dose for treatment of Graves' Disease which will both alleviate the disease and avoid induced hypothyroidism.

Technical Approach: Study of I-131 uptake and I-127 content of hyperthyroid glands in freshly diagnosed patients with Graves' Disease.

Progress and Results: No appropriate patients were found for this study in the past year. This was due to the fact that all patients were either on medical treatment at the time they presented to this clinic or had to be placed on medical treatment before they could be reasonably studied. This interfered with the initial objectives of the study and for that reason the study has been terminated.

Conclusions: None.

Funding Requirements: N/A.

Personnel: N/A.

Equipment: N/A.

Supplies: N/A.

Travel: N/A.

Pucliations: N/A.

Work Unit No: 4518

Title of Project: Clinical Evaluation of  $^{99m}$ Tc Electrolytically labeled  
human serum albumin

Investigators:

Principal: Merrill C. Johnson, Col, MC

Progress and Results: This study has been postponed pending receipt  
of a low voltage power supply.

Conclusions: None. Study will be continued on receipt of power supply.

Findings/Requirements: None

Personnel: N/A

Equipment: N/A

Supplies: N/A

Travel: N/A

Other: N/A

Work Unit No.: 4601

Title of Project: Participation in the National Cooperative Study of Early Hodgkin's Disease

Investigators:

Principal Investigator: George B. Hutchison, M.D.

Associate Investigator: Hans Blom, M.D. and Thomas J. Stoffel, M.D.

Objectives: To study the effects on the survival of patients with early staged Hodgkin's Disease of differing irradiation volumes.

Technical Approach: This clinical study was randomized and prospective comparing localized irradiation to clinically involved region as compared to extended-field radiotherapy.

Progress & Results: An interim report was presented at the last meeting of all the participating institutions held in Chicago, July, 1976. While there were many more local recurrences in the patients receiving localized treatment, there were a few more deaths in the extended-field treatment group.

Entry of patients into this study was terminated in 1971. At the meeting mentioned above it was decided that follow-up of 10 years or more might be needed to conclude the study. The survival of both groups is substantially better than projected at the outset.

Conclusions: To date, comparison of localized fields to extended-field therapy of early Hodgkin's Disease has not shown a clear superiority of either technique within the follow-up period so far achieved. The study suggests that extensions following extended field therapy may routinely carry a poor prognosis but that local extensions following local field therapy may not have this grave significance.

Funding Requirements:

- a. Personnel: None
- b. Equipment: None
- c. Supply: None
- d. Travel: \$4,800

Publications:

1. Hutchison, George B., Progress Report. Hodgkin's Clinical Trial, 1972, National Cancer Institute Monograph, No. 36, International Symposium on Hodgkin's Disease, Pgs. 387-393.
2. Nickson, James J., Hutchison, G. B., Hodgkin's Disease Clinical Trial. Sixth National Cancer Conference Proceedings, 1968., pgs. 77-81.

3. Nickson, James J., Hutchison, G.B., Extensions of Disease, Complications of Therapy, and Deaths in Localized Hodgkin's Disease; Preliminary Report of a Clinical Trial. The American Journal of Roentgenology, Radium Therapy and Nuclear Medicine, Vol. CXIV, No. 3, March, 1972, pgs. 564-573.

Funding Requirements:

Authorized FY 76: \$4,800

FY 77:

Travel: \$4,800

Work Unit No.: 6001

Project Title: Study of Effect of Betadine Bath  
on T<sub>4</sub> Levels in the Newborn.

Principal Investigator: LTC R. D. Landes, MC  
Department of Pediatrics

Status: The status of this project is not clear. Annual Progress Report by the Principal Investigator has not been submitted. At the 29 September 1976 meeting of the Clinical Investigation Committee meeting, termination of this project was considered. The Committee did not terminate the project at the request of the representative from the WRAIR.

Work Unit No: 6002

Title of Project: Evaluation of Blood Volume Expansion as an Adjunct to Therapy of Hyaline Membrane Disease

Investigators: Principal: Richard D. Landes, LTC, MC  
Associate: Bernard A. Wiggins, CPT, MC

Objectives: The objective of this study was to determine whether early detection of hypovolemia by measuring blood volume and early correction of hypovolemia in neonates with hyaline membrane disease would lead to increased survival.

Technical Approach: The plan was to adapt a method of using Technetium-labeled Red Blood Cells developed by William C. Eckelman et al (The American J. Roentgenology, Radium Therapy and Nuclear Med., Vol. 118, Aug 75) for use in measuring red cell volume in neonates. This would have allowed measuring red cell volume accurately with a minimal, safe radiation exposure to the newborn patient.

Patients were to have been divided into 2 groups; 1 control group who received no initial blood transfusion and 1 transfusion group who would have received any early blood transfusion to correct the blood volume to the normal 90-100 ml/kg of body weight. All infants were to be closely monitored and treated in the same manner otherwise for hyaline membrane disease.

The two study groups were to be compared at the end of the study to determine whether early correction of blood volume increased survival rates.

Progress & Results: No progress has been made in this study because of difficulties in preparation of the Technetium-labeled red blood cells. Since the labeling process involves the combining of  $^{99m}$  Technetium with a Tin-chloride solution to facilitate red-cell labeling which could not be performed at WRAMC, we were dependant upon either Dr. Eckelman's laboratory at Washington Hospital Center or the Radio-pharmacy at NIH to prepare this solution for us. After submitting this protocol the law changed regarding isotopes, so that isotope solution came to be classified as drugs and required licensing. Because of the above, Dr. Eckelman's laboratory was unable and unwilling to prepare our  $^{99m}$  Technetium-Tin-chloride solution. The radio-pharmacy at NIH refused to approve any such solution they may prepare for human use, plus there is an additional problem of transporting the isotope across state lines i.e. Maryland to District of Columbia.

In view of the above technical problems, it does not appear that this study can be performed at this time.

Conclusions: It is requested that because of the above technical difficulties that this study be suspended at this time. If we are eventually able to overcome the obstacles in the future, the Protocol will be submitted for consideration. It is requested that all equipment relating to this study be transferred to the Department of Pediatrics for Clinical use in the interim.

Funds Utilized: FY 76-7T - none

Funds Required: FY 77 - None

Publications: None

Type of Report: Terminated

Work Unit No.: 6009

Title of Project: Clinical and Laboratory Investigation of  
Meningeal Leukemia

Investigators:

Principal: Frederick B. Ruymann, M.D. LTC MC

Associate: Bill Cunningham, M.D. LTC MC

Objectives: Comparison of biochemical and immunological variables  
in the cerebrospinal fluid of patients with Acute  
Lymphocytic Leukemia under treatment with intrathecal  
methotrexate vs. intrathecal methotrexate and CNS ir-  
radiation.

Technician Approach: Patients with leukemia under the direct and  
consultative care of the Pediatric Hematology/Oncology  
Service will be used as the primary source. Additional  
patients will be studied. Clinical histories, flow  
sheets, and summaries are already maintained by child  
and adult oncologists at present. Particular note will  
be made of the time of diagnosis, length of remission,  
past history of CNSL, and therapy for CNSL and type of  
prophylactic CNSL treatment. As new patients are acquired  
on #7411 they will be followed along. Spinal fluid  
and blood specimens will be obtained. Patients in  
clinical remission will have confirmatory bone marrow  
aspirated and spinal fluid analysis every two months.  
This consent will be obtained on all procedures. This  
protocol involves no procedures beyond those already  
recommended by ALGB.

Progress & Results: Satisfactory preparations of spinal fluid  
cytology were obtained using the Cyto-Centrifuge.  
The technique was modified to utilize AB negative  
serum. Technical factors of centrifuge speed were  
solved.

In a recent patient (K.K) previously diagnosed as  
Ewing's Sarcoma, the spinal fluid cytology suggested a  
different diagnosis. Following this lead, the patients'  
diagnosis was changed to Non-Hodgkins Lymphoma.

A second patient, G.W., was detected to have meningeal leukemia when ordinary CSF examinations were normal. This early and unexpected detection lead to aggressive high dose and intrathecal methotrexate therapy which secured a remission.

Conclusions: (1) Examinations of CSF cytology utilizing the cytocentrifuge has significantly improved the sensitivity and accuracy in detecting meningeal involvement with leukemia and solid tumors.

(2) Immunological aspects are presently beyond the capability of this laboratory. We will continue to use the cytocentrifuge on CSF specimens, but will save the CSF specimens for later analysis.

Funds Utilized, FY-77: \_\_\_\_\_

Funding Requirements, FY-77:

Personnel: Doris Burgess, GS-9, about 9 months over the next two years.

Equipment: None

Supplies: \$550 reagents, histochemical stains, LDH, isoenzyme reagents

Travel: None

Publications: None applicable to this project

Type of Report: Interim

Work Unit No.: 6010

Title of Project: Urinary Catecholamine Determination in Childhood Malignancy.

Principal Investigator: LTC Frederick B. Ruymann

Status: Several requests for the Annual Progress Report on this project have been ignored. The Clinical Investigation Committee in a meeting on 29 September 1976, terminated this study. Funds to support this effort are withdrawn. The Technician will be assigned other duties within the Service for four months of the year.

Work Unit No.: 6013

Title of Project: Evaluation of Four Modes of Therapy of Reye's Syndrome, Acute Encephalopathy with Fatty Infiltration of the Viscera: A Multi-Hospital Study

Investigators:

Principal: William J. Oetgen, M.D., MAJ, MC

Associates: James E. Shira, M.D., COL, MC  
Frederick B. Ruymann, M.D., LTC, MC  
Lawrence R. Hyman, M.D., MAJ, MC

Objective: The pediatric literature is replete with anecdotal reports of various therapeutic measures for Reye's syndrome. Survival rates vary from 0% to 90% for similar regimens. There has been no controlled, randomized study of these therapeutic measures. The objective of this study is to determine the effectiveness of four modes of therapy for Reye's syndrome (1) supportive care alone, (2) supportive care with exchange transfusions, (3) with peritoneal dialysis and, (4) with glucose and insulin. To our knowledge, this is the only such study being conducted.

Technical Approach: Informed consent of the patient's parents will be obtained, and the protocol attached will be followed.

Progress & Results: Since approval was granted for this protocol, no patient has been admitted to this hospital who has been appropriate for admission to the study. The protocol remains active and will be used when a patient with Reye's Syndrome is admitted.

Conclusions: None at present

Publications: None to date

Work Unit No.: 6014

Title of Project: Granulocyte Transfusion by Two Methods:  
Continuous Flow Centrifugation and Filtration  
Leukaphoresis

Investigators:

Principal: Frederick B. Ruymann M.D., LTC MC

Associate: Alan Mease, M.D. MAJ MC

Technical Assistance: Doris Burgess - Medical Technician

Objectives: Granulocyte transfusion has been used at Walter Reed Army Medical Center since 1969, utilizing the IBM-NCI Cell Separator. In this program selected patients with Neutropenia and suspected or proven sepsis have received granulocyte transfusions. The results of this work have recently been accepted for publication.<sup>1</sup> Our present study endeavors to compare the continuous flow centrifugation methods (IBM-NCI Cell Separator) with the newer method of filtration leukaphoresis. This latter method if comparable to continuous flow centrifugation would be saving considerable technician time.

Technical Approach: A planned comparison of donor reactions, clinical response in recipients, granulocyte yield and neutrophil Adenosine Triphosphate (ATP) content is planned utilizing two methods of granulocyte collection.

Clinical variables of febrile response, negativity of positive blood cultures, and survival will be observed. The number of granulocytes, relative recovery one hour after infusion, and ATP concentration will be measured. The ATP concentration is an index of all viability and should show correlation with the one hour recovery values.

Progress & Results: (1) During the past year Ms. Burgess has been trained in the use of filtration leukaphoresis by the Fenwall Corporation. She is now fully qualified to run the IBM-NCI Cell Separator and perform leukaphoresis.

(2) Equipment and reagents for performing ATP determinations on leukocytes were acquired. Preliminary determinations showed that granulocyte ATP concentration from the peripheral blood were limited by the relatively small number of granulocytes.

(3) Lymphocytes and granulocytes were successfully separated from whole blood by defibrination and ficoll-gradient. A pure granulocyte preparation could not be obtained.

(4) Although a comparison of the two methods of collection could not be used granulocyte transfusions were given to eight children in the past fiscal year. A clinical response with survival beyond a week was observed in 75% of the children.

Conclusions: (1) There are no major technical impediments to the comparison of these two granulocyte collection methods. Recent literature suggests that the granulocyte function of filtration acquired white cells is inferior to cells harvested by centrifugation. This finding would not deter the value of this study, since by design the patient would receive both types of white cells on successive days.

(2) Granulocyte transfusion is highly efficacious in the neutropenic child who has a relatively small blood volume and in whom recovery from an aplastic marrow is anticipated. The procedure has limited value in those individuals with overt relapse and resistance to Cancer Chemotherapy.

Funds Utilized FY-76: \$5,100.00

Funding Requested FY-77: \$5,000.00

Personnel: GS-9 Technician (currently employed) - 3 month/year  
Equipment: Consumable Supplies \$600.00

Publications: Maybee, D.A., Millan, A., and Ruymann, F.B.,  
Granulocyte Tranfusion in Childhood, So. Medical  
Journal

Type of Report: This is an interim report, two more years are anticipated to bring this study to conclusion.

Work Unit No.: 7105

Title of Project: Study of CEP Responses in Pediatric Epileptic Patients  
Before and After Withdrawal of Anticonvulsants

Investigators:

Principal: Archer D. Huott, COL, MC

Associate: Charles L. Walters, MAJ, MC

Objectives: To develop technique for prediction of favorable prognosis  
regarding pediatric epileptic patients after anticonvulsant  
drug withdrawal.

Technical Approach: This study is essentially a comparison of a child's  
prior CEP's before and after withdrawal of anti-  
convulsant drugs in those epileptic patients meeting  
the following criteria:

- 1) Onset of idiopathic epilepsy afebrile seizures  
below the age of 12.
- 2) Freedom of minor or major seizures for a period  
of two years.
- 3) Normal awake EEG with sleep, hyperventilation,  
auditory and stroboscopic activation.

It is felt that by such a study those patients who  
will eventually relapse will be detected early enough  
to reinstitute therapy and prevent the resumption of  
clinical seizures.

Progress & Results: The past year has been spent debugging currently  
present equipment. Data acquisition on our equipment  
is unsatisfactory for compilation at A.F.F.R.I.,  
therefore new equipment from A.F.F.R.I. is currently  
being installed to use in this project.

Funds Utilized FY-76: None, however will require funds during June 76  
of approximately \$1322.00 for ten hours computer  
time.

Funding Requested FY-77: \$5280.00 for 40 hours of computer time at  
A.F.F.R.I. (@ \$132/hr)

Publications (FY-76): None

Type of Report: Interim

Work Unit No.: 7107

Title of Project: The Effects of a Unilateral Vascular Accident Within the Cerebral Cortex Producing Hemiplegia Upon Certain Psychological Functions.

Responsible Investigators:

Division Director:	COL Harry C. Holloway, M.D.
Department Chief:	David H. Marlowe, Ph.D.
Investigators:	MAJ David W. Pearson, M.D. CPT Ronald Pfeifer, M.D.

Objectives:

1. To describe linguistic and spatial-perceptual functioning in unilateral CVA's who have no clinically defined aphasia or spatial perceptual problems 3-6 months post CVA;
2. To correlate linguistic functions and spatial-perceptual abilities with the locus of the lesion;
3. To document changes in linguistic function and spatial-perceptual functioning over time in patients who have had a unilateral CVA;
4. To help in understanding the process within the central nervous system that are related to language and spatial perceptual functioning.

Technical Approach:

10 patients who have undergone a left sided cerebral vascular accident who do not carry the clinical diagnosis of aphasia but who have some neurological sequelae will be defined. 10 patients who have undergone a comparable right sided cerebral vascular accident with only minimum residual impairment will be defined. 10 patients who have undergone a traumatic lower limb amputation will also be defined. Patients will be selected on the following seven criteria:

1. First cerebral vascular accident.
2. No uncontrolled medical complication.
3. Only right sided or left sided involvement.
4. No residual aphasia.
5. Forty to eighty years old.
6. At least an eighth grade education
7. No psychosis

Within the requested increase in budget, each patient will be given a computer assisted transaxial tomogram to define the locus and extent of the cerebral lesion three months after the CVA. A preselected battery of psychological tests will be administered.

These tests are:

1. The Columbia Mental Maturities Test
2. Kohs Block Design
3. Revised Minnesota Paper Form Board Test
4. Three Card Thematic Apperception Test
5. Cloze Technique
6. Make A Sentence Test
7. Psycholinguistic Tasks presently being developed.

Progress Report and Future Directions:

A lack of appropriate case materials and the introduction of other organizational demands have resulted in essentially no significant progress on the research project.

The principal investigator has left the U.S. Army. Given the minimal progress on work unit 7107, it is terminated.

Work Unit No.: 7108

Title of Project: Visual Evoked Responses in the Diagnosis of Multiple Sclerosis

Investigators:

Principal: Archer D. Huott, COL, MC

Associate: Charles L. Walters, MAJ, MC

Objectives: To arrive at an objective criterion for retrobulbar neuritis.

Technical Approach: Flashes of light are presented to each eye independently and patient's CEP from recording electrodes over the occiput are analyzed by computer technology.

Progress & Results: None. Past year spent acquiring additional apparatus and equipment necessary to insure an artefact-free recording. Newly arrived equipment should eliminate these problems.

Conclusions: None.

Funds Utilized: None, however cost of computer time at AFIP may be required during June 1976.

Funding Requested FY-77: Same as FY-76 (see #7105).

Publications: None.

Type of Report: Interim.

Work Unit No.: 7109

Title of Project: The Application of Somatosensory Spinal Evoked Response in Spinal Cord Pathology.

Investigator: Archer D. Huott, COL, MC

Objectives: To correlate clinical and evoked level of lesion with finding at operation. Also to obtain normative data.

Technical Approach: Somatosensory input in the form of electrical shocks to the nerves of the leg and pickup of these potentials over the spinal cord at various levels and also over the somatosensory cortex. This analysis utilizes computer technology.

Progress & Results: The past year has been spent in debugging current apparatus to generate shocks and has been hampered with artefact in the recording area. Newly arrived equipment should eliminate these problems.

Funds Utilized FY-76: May require funds for computer time at A.F.F.R.I. during month of June (approximately 10 hours @ \$1322.00)

Funding Requirements FY-77: Same as FY-76.

Publications: None.

Type of Report: Interim.

Work Unit No.: 7110

Title of Project: System Analysis Applied to Clinical Neurology

Investigators:

Principal: COL Darrell S. Buchanan, MC

Progress & Results: This project has not yet been initiated. The clinical components of the project which are to be accomplished at WRAMC are dependent on the overall approval and funding of the basic project by NIH.

Work Unit No.: 7203

Title of Project: Use of Biofeedback Techniques in the Treatment of Migraine Headaches

Investigators:

Principal: Juan M. Garcia, MD, MAJ, MC

Objectives: To compare results obtained by applying recently developed electromyographic and temperature biofeedback techniques in the treatment of migraine headaches with those obtained from conventional, currently employed medical management of migraine headaches.

Technical Approach: Statistical comparisons will be conducted among three groups of patients suffering from migraine headaches. Fifteen patients will be treated with electromyographic biofeedback and 15 patients will receive currently accepted medicinal treatment for migraine headaches (i.e., Cafecot). Informed consent forms will be obtained from all patients admitted to the study.

Progress & Results: The project is still pending since the Principal Investigator is working on higher priority projects at the Departments of Military Psychiatry and Military Medical Psychophysiology, WRAIR.

Conclusions: N/A.

Funds Utilized FY 76: None.

Funding Requested FY 77: None.

Publications: None.

Type of Report: Interim.

Work Unit No.: 7205

Title of Project: Spanish-American Patients: Considerations for Their Psychiatric Assessment

Investigators:

Principal: Juan M. Garcia, MD, MM, MC

Objectives: (1) To determine whether there are differences in determination of the amount of psychopathology in patients whose native language is Spanish when they are interviewed in English and in Spanish; (2) To determine whether there are diagnostic differences between English and Spanish speaking raters; (3) To determine the extent to which language competence and fluency in English contributes to differences in psychopathology observed (if any); (4) To better understand the roles of other factors such as positive and negative rater prejudice, raters' ideological orientation, and attitudes of patient before and after interview; and (5) To compare differences of personality profiles of the MMPI when given in English and Spanish.

Technical Approach:

1. Videotaped interviews of hospitalized Puerto Rican psychiatric patients will be conducted in English and Spanish. These will then be assessed and rated independently by English and Spanish speaking raters.
2. Questionnaires regarding racial and ethnic attitudes and prejudices will be administered to both the patients and the raters.
3. MMPIs will be administered to patients both in English and in Spanish.
4. Statistical analyses will be performed on the data obtained from the three sources mentioned above.

Progress & Results: The project is still pending since the Principal Investigator is working on higher priority projects at the Departments of Military Psychiatry and Military Medical Psychophysiology, WRAMC.

Conclusions: N/A.

Funds Utilized FY 76: None.

Funding Requested FY 77: None.

Publications: None.

Type of Report: Interim.

Work Unit No.: 7207

Title of Project: Psychiatric Inpatients' Reactions to Rectal Examinations by Treating Psychiatrists

Investigators:

Principal: William Logan, MD, MAJ, MC

Objectives: To assess reactions of psychiatric inpatients to rectal examinations performed by their assigned physician.

Technical Approach: Utilization of instruments to assess physicians' observations of patients' reactions and self-reported patient reactions.

Progress & Results: The project has been terminated due to the departure of the Principal Investigator and there has been no data collection.

Conclusions: None.

Funding Requirements: None.

Funding Requested FY 77: N/A.

Publications: None.

Type of Report: Termination (see Progress & Results above)/

Work Unit No.: 7211

Title of Project: Discharge Recommendations and Morbidity in Psychotic Servicemen

Investigators:

Principal: Donald W. Morgan, MD, COL, MC

Objectives: To compare the morbidity of psychotic servicemen transferred to VA hospitals with those discharged to their own care during the first year following release from WPAAC.

Technical Approach: Follow-up of two groups of 100 patients each--half of whom have been referred to the VA hospital and the other half who were transferred to a VA hospital on an inpatient basis. All patients will have been rated on a standard mental status form and will have had an initial MMPI and complete social history. These will be used to compare the patients within the two groups as to the amount of pathology and morbidity. The subjects will be contacted at 3, 6, 9 and 12 months following discharge from the hospital. Outcome criteria will include number of days in the hospital, number of working days, total income, plus their functioning in areas of recreation and interpersonal behavior. The number of contacts with mental health professionals and their treatment course including medications will also be gathered. Informed consent forms will be obtained on all patients admitted to the study.

Progress & Results: To date, 11 subjects have been entered into the study and some psychological tests have been administered.

Conclusions: None.

Funding Requirements: None.

Funding Requested FY 77:

Publications: None.

Type of Report: Interim.

Work Unit No.: 8026

Title of Project: Effect of Sharpening Stones on Kirkland Gingivectomy Knives Studies under the Scanning Electron Microscope.

Investigators: Principal: Charles J. Antonini MAJ DC  
Associates: Ronald Van Swol COL DC  
John Brady COL DC

Objectives: A comparison of three commonly used sharpening stones will determine which stone will better restore the Kirkland cutting edge.

Technical Approach: 10 Kirkland #15 and #16 (5 each) gingivectomy knives will be used. The sharpening will be accomplished by the chief of periodontal services at Walter Reed Army Medical Center. The scanning electron microscope prints will be read by the chief of biophysics, United States Army Institute of Dental Research. The gingivectomy knives will be randomly selected by placing a standard order for these instruments. Preparation: The instruments will be prepared for the scanning electron microscope. This will consist of (1) cutting off the shaft from the body, (2) honing down the shaft to reduce the bulkiness for reception in the scanning electron microscope chamber. The reduced specimen's shaft will be received by a Morse holder for surgical use. One side of the bowed cutting edge will be marked by an electrical engraver. This marked side will be investigated. The area between the two marks will be scanned to select a representative point that will be photographed. A customized jig will be constructed to mount the instrument on the aluminum tab. This will insure observation of all edges from the same angle. The magnification will be 500x. All new blades will be photographed, these prints serving as the base line. The instruments will be autoclaved and photographed, then autoclaved again and used in a maxillary gingivectomy procedure (around 5-7 teeth). Three randomly selected blades will be photographed, this indicating the degree of dullness. Five knives will then be resharpened by a rotary Arkansas stone (Hu-Friedy #301) and five knives by a rotary ruby grit stone. All resharpened knives will be photographed.

Data Analysis: The level of measurement will be nominal. Contingency co-efficient will determine the degree of correlation. The overall test of significance will be determined by the chi-square statistical test.

**Progress and Results:** Scanning electron microscope photographs have been taken of the Kirkland gingivectomy knives from two views. One view was from a lateral aspect of the edge and the second a straight-on view of the cutting part. Photographs were taken of both views at 500x and 1000x. Twenty factory knives, 10 autoclaved knives and 10 resharpened knives have been photographed. The remaining 10 knives are now being processed.

**Conclusions:** The factory sharpened knives exhibited two types of cutting edges. Both types had wire edges, which is a metallic extension of two surfaces. One type exhibited a uniform meeting of both surfaces that formed the wire edge. The second type exhibited a wire edge that was deformed toward one surface. Steam autoclaving did not deform the cutting edge. Dullness was characterized by complete metallic deformation of the wire edge and plastic deformation of adjacent metallic surfaces or just a wire edge deformation. Hand resharpened knives' wire edges did not have the uniformity that was noted in the factory sharpened knives.

**Funding requirements:** None.

**Type of Report:** Completed

Work Unit No: 8027

Title of Projects: Clinical Evaluation of Freeze-dried Bone Allographs  
in the Treatment of Severe Periodontal Osseous Defects

Investigators:

Principal: Ronald L. Van Swol, D.D.S.  
Chief, Periodontia Service, WRAMC

Associate: All Residents assigned to the Periodontia Service

Objectives: To evaluate the effectiveness of freeze-dried human bone allographs in the treatment of periodontal osseous defects.

Technical Approach: Consenting patients with large, severe periodontal osseous defects will be treated, using freeze-dried human bone allographs. Full thickness buccal and lingual flaps are developed in the surgical area, all granulation tissue is removed from the defect, the root surface is cleaned, and the osseous defect filled with the allograph material. The flaps are then repositioned and sutured to place. The surgical field is then covered with periodontal dressing and postoperative instructions given. The patient is seen in one week for suture removal and periodontal dressing change. At the two weeks postoperative time frame, the dressing is removed and home care instructions are given. The patient is then seen every 3 months for re-evaluation of the grafted area. At one year post-operatively, the area is re-entered for final evaluation and further grafting, if needed.

Progress and Results: During FY-76, 15 severe periodontal osseous defects were grafted. No complications were encountered in any of the cases, and good documentation was acquired in all instances.

Conclusions: During FY-76, we grafted 15 periodontal osseous defects. Our overall response has been of greater than 50% osseous regeneration, which has been very gratifying to the principal investigator.

Funds Utilized, FY-76: None

Funding Requirements, FY-77: None, the personnel, equipment and staff (D.D.S.) of the Periodontia Service, Dept of Dentistry, Walter Reed Army Medical Center will be utilized.

Publications: None

Type of Report: Interim.

Work Unit No.: 9001

Title of Project: Factor VIII Activity/Antigen Ratio in von Willebrand's Disease. I. Epinephrine Effect.

Investigators:

Principal: MAJ David J. Ahr, MC

Associate: Frederick R. Rickles, M.D., VA Hospital, Newington CT; Leon W. Hoyer, M.D., University of Connecticut Health Center, Farmington CT

Objectives: To determine the relationship of factor VIII procoagulant activity ( $VIII_{ACT}$ ), factor VIII antigen levels ( $VIII_{AGN}$ ), and the antibleeding factor or von Willebrand factor ( $VIII_{VWF}$ ) in patients with von Willebrand's disease (VWD) and other molecular variants of factor VIII metabolism.

Technical Approach: It is known that the factor VIII molecule either represents several molecules bound together on a single carrier protein with separate activities or a single macromolecule (est. m.w.  $2 \times 10^6$ ) with subunits containing the separate activities. Recent evidence has suggested that salt dissociation (high molarity) of semi-purified factor VIII produces a large molecular weight piece and a smaller molecular weight piece. Utilizing the radioimmunoassay for  $VIII_{AGN}$  described by one of us (Dr. Hoyer) and the assay for  $VIII_{VWF}$  described by us (Weiss, Hoyer, and Rickles, J Clin Invest 52: 2708, 1973), we hope to define the location of these activities on the molecule and monitor the effects of such agents as epinephrine on release of storage material. Recent work suggests that the high molecular weight piece contains  $VIII_{AGN}$  and  $VIII_{VWF}$  but little or no  $VIII_{ACT}$  while the low molecular weight piece contains only  $VIII_{ACT}$ . Obviously, the nature of the so-called von Willebrand "kick" (that is, the  $VIII_{ACT}$  that is seen following either stress or transfusion) may be better defined by analyzing the material for  $VIII_{AGN}$  and  $VIII_{VWF}$ . It is conceivable (and likely) that the "kick" represents only low molecular material without  $VIII_{VWF}$ . Such evidence would explain the inability of transfusion to correct the bleeding time in many patients with von Willebrand's disease.

Progress and Results: Five volunteers with von Willebrand's disease of varying severity were studied. No complications of epinephrine infusion were encountered. Mean pulse rate increase of 33 beats/minute, mean systolic blood pressure increase of 39 mm Hg, and a widened pulse pressure were observed. Symptoms during 30 minutes epinephrine infusion to a total dose of 0.0042 mg/kg were limited to mild anxiety palpitations without dysrhythmia, and peripheral vasoconstriction. All symptoms subsided within 15 minutes after epinephrine infusion was discontinued.

Analysis of post-infusion plasma samples reveals:

- (1) Epinephrine infusion causes variable increases in the components of the factor VIII (antihemophilic factor) complex in patients with von Willebrand's disease.
- (2) The increase in antihemophilic factor procoagulant activity ( $VIII_{AHF}$ ) was greater than that of factor VIII antigen ( $VIII_{AGN}$ ) and von Willebrand factor activity ( $VIII_{VWF}$ ) in two patients with moderately severe von Willebrand's disease. Similar increases in the three factors were demonstrated in two other less severely affected patients. A four- to ten-fold increase in factor VIII-related properties was identified in each of these individuals after infusion.
- (3) One patient with severe von Willebrand's disease demonstrated no increase in the factor VIII-related properties during two infusions of epinephrine.
- (4) Bleeding times were normalized or remained normal in the two patients whose  $VIII_{VWF}$  was greater than 25 units/100 ml. The bleeding time remained prolonged in those three patients whose  $VIII_{VWF}$  levels remained below this concentration.
- (5) The increase in procoagulant activity was transient in all patients with  $T_{1/2}$  values estimated to be between 0.8 and 3.4 hours.
- (6) The rapid increase of all three factor VIII-related properties after epinephrine infusion suggests that epinephrine stimulates the release of preformed factor VIII rather than new synthesis.
- (7) An estimate of the molecular size of the "stimulated  $VIII_{AHF}$ " is obtained by agarose gel chromatography, and sucrose density gradient ultracentrifugation suggests an apparent molecular weight of greater than  $1.0 \times 10^6$  daltons. This is identical to that reported for normal plasma  $VIII_{AHF}$ .

Conclusions: This study has provided useful data in explaining the observed variation in factor VIII-related properties and bleeding tendency in patients with VWD. It strongly suggests that catecholamine or other hormonal therapy may be beneficial to some patients with mild to moderately severe disease. No benefit would be expected for patients with severe VWD. Further studies of this type should continue to provide pertinent clues to the molecular abnormalities of factor VIII in VWD and hemophilia K. This study as written should be terminated.

Funds Utilized, FY 76: \$500

Funding Requirements, FY 77: None.

Publications: Frederick R. Rickles, Leon W. Hoyer, Margaret E. Rick,  
and David J. Ahr: The effects of epinephrine infusion  
in patients with von Willebrand's disease. J. clin.  
Invest., in press (to be published June 1976).

Type of Report: Completed.

Work Unit No.: 9002

Title of Project: Effects of Oral Contraceptives on Platelet Function and Magnesium Metabolism

Investigators:

Principal: MAJ David J. Ahr, MC  
Associate: MAJ Alberto Avilas, MC

Objectives: To determine if enhanced platelet reactivity to various in vitro stimuli and alteration of serum and urine magnesium concentrations can be observed following exposure to oral contraceptive agents, and if tests of platelet reactivity are sufficiently sensitive and specific to identify individuals among oral contraceptive users at increased risk of developing thromboembolic disease.

Technical Approach: Forty women never previously exposed to oral contraceptives or other exogenous hormones will be sought among patients coming for contraceptive advice to the Department of Obstetrics and Gynecology, WRAMC. Twenty women will use chemical contraception and 20 will use the diaphragm or rhythm method. No other medication will be ingested during the study period. Standard tests of platelet function (to include aggregation response to dilute aggregating agents and platelet factor III release) as well as routine coagulation factor analysis, and serum and urine magnesium concentrations will be performed three times per menstrual cycle for three menstrual cycles in all patients.

Progress and Results: Preliminary studies and validation of procedural methods for identifying platelet hyperreactivity and identifying patients at increased risk for thrombosis have been gathered from a similar study of patients with severe vascular disease and normal subjects. Due to the transfer of the associate investigator during FY 76, the patient selection and followup became impossible. No randomized studies were attempted, and this study should be terminated.

Conclusions: None

Funding Utilized, FY 76: None

Funding Requirements, FY 77: None

Publications: None

Type of Report: Terminated

Work Unit No.: 9004

Title of Project: Prevention of transfusion hepatitis with hyper-immune HBAb serum gamma globulin

Investigators:

Principal: Marcel E. Conrad, M.D., Professor of Medicine  
Director, Division of Hematology/Oncology  
University of Alabama in Birmingham

Associate: MAJ Robert G. Knodell, M.D., MC  
Allen L. Ginsberg, M.D., Asst. Professor of Medicine,  
George Washington University  
LTC E. Patrick Flannery, M.D., MC,  
Letterman Army Institute of Research

Objectives: To determine if gamma globulin with a high HBAb titer provides protection against transfusional hepatitis and if transfused blood with HBAg only by radioimmune assay but not by other methods of testing causes hepatitis.

Technical Approach: During recent years, there has been a high incidence of hepatitis among patients undergoing cardiac bypass surgery at WRAMC. Estimates of the incidence of hepatitis in this group (based on the detection of elevated transaminase determinations 3 months after surgery) are 10% to 20% of patients. It is believed that this is caused by the requirement of the use of many pints of blood and blood products from multiple donors in these patients.

From August 1972 through December 1974, all volunteers who underwent cardiac bypass surgery received a 10-ml injection of either high titer HBAb gamma globulin, conventional gamma globulin, or an albumin placebo solution. These injections were administered double blind under code. Blood was drawn from the volunteers before gamma globulin injection, weekly after surgery while the patient was hospitalized, and at 3, 6, and 9 months after surgery. The blood specimens were tested for HBAg, HBAb, SGOT, SGPT, and serum bilirubin determinations. In addition, a history was obtained from each patient at intervals after surgery. All blood used for transfusion was tested by radio-immune assay for HBAg and for HBAb.

The biologic materials used in this study included a high titer HBAb lot of gamma globulin prepared by the Massachusetts State Laboratories, a lot of gamma globulin used in 60,000 soldiers in Korea, and a placebo solution used in 40,000 U.S. soldiers without known complications in Korea. All solutions were tested in accordance with U.S.P. regulations.

Progress and Results: Three hundred fifty-two patients were enrolled in the study when acquisitions terminated in December 1974, and followup of all patients was completed during September 1975. Complete followup was obtained in 279 patients who received blood transfusions while undergoing cardiac surgery. Each subject of the study received either high titer (anti-HB<sub>S</sub>) or normal gamma globulin or a placebo, and each was followed for 9 months. Seventeen percent (47) of these patients developed transaminase elevations 14-180 days postoperatively; 10 of these patients were icteric. Serologically, only 3 of these patients had hepatitis B, 3 had cytomegalic inclusion virus infection, and none had hepatitis A. The risk of developing posttransfusion hepatitis seemed greater in patients transfused with blood containing anti-HB<sub>S</sub>. A decreased morbidity was observed in patients who received either high titer or normal gamma globulin. High titer gamma globulin seemed to decrease the transmission of hepatitis B, while normal gamma globulin and placebo injections did not. No adverse effects were observed in the gamma globulin-treated patients. The results of these studies show significant protection against developing posttransfusion hepatitis with prophylactic gamma globulin administration and will have important bearing on practices within blood banks in the United States as well as provide support for prophylactic gamma globulin treatment of patients receiving multiple blood transfusions.

Conclusions: The study has shown a significant incidence of non-A and non-B hepatitis in patients receiving multiple blood transfusions at cardiac surgery. The study indicates that blood containing Australia antibody (anti-HB<sub>S</sub>) has a higher probability of causing hepatitis in blood recipients than blood not possessing this antibody. The study shows that gamma globulin has a salutary effect in reducing the morbidity and transmission of hepatitis in patients receiving blood transfusions.

Funds Utilized, FY 76: None

Funding Requirements, FY 77: None

Publications:

R. G. Knodell, M. E. Conrad, J. L. Dienstag, and C. J. Bell: Etiological spectrum of posttransfusion hepatitis. *Gastroenterology* 69: 1278-1285, 1975

M. E. Conrad and R. G. Knodell: Viral hepatitis. *JAMA* 233: 1277-1278, 1975

R. G. Knodell, M. E. Conrad, A. L. Ginsberg, E. P. Flannery, and C. J. Bell: Efficacy of prophylactic gamma globulin in prevention of non-A, non-B posttransfusion hepatitis. *Lancet* I: 557-561, 1976

Type of Report: Completed

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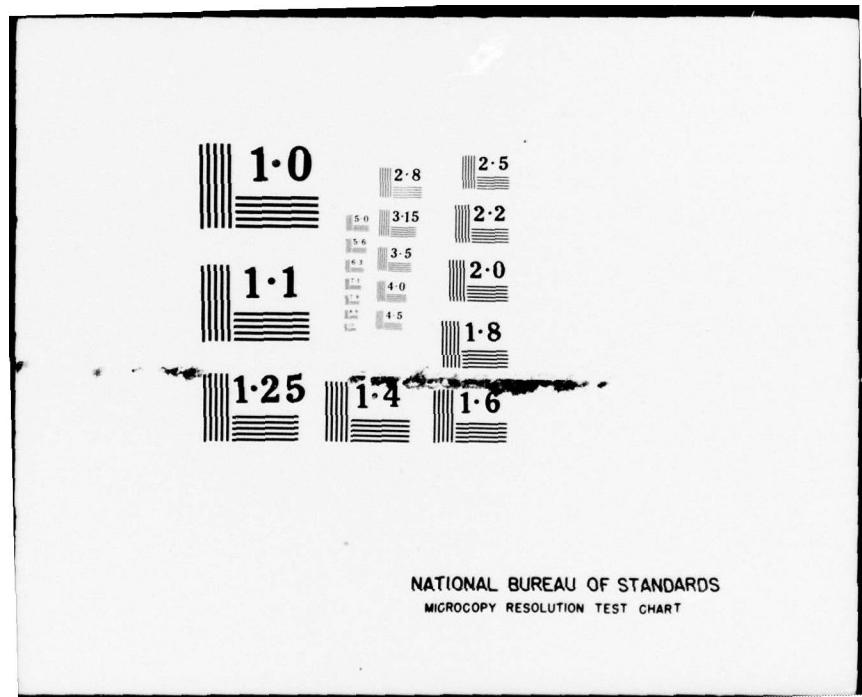
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Work Unit No.: 9008

Title of Project: Compositional abnormalities in cirrhotic bile

Investigators:

Principal: MAJ Robert G. Knodell, M.D., MC

Associate: MAJ M. Dean Kinsey, M.D., MC

MAJ Edgar C. Boedeker, M.D., MC

Objectives: To elucidate mechanism for abnormal secondary bile acid metabolism in alcoholic cirrhotic patients and to study biliary lipid secretion and bilirubin conjugation in patients with alcoholic cirrhosis.

Technical Approach: Multiple analyses of bile obtained by biliary drainage and qualitative and quantitative analysis of fecal and urinary bile acids using thin layer and gas-liquid chromatography.

Progress and Results: Seven patients with biopsy-documented cirrhosis have been studied. A marked decreased in 7 $\alpha$ C dehydroxylation of primary bile acids has been documented both *in vivo* and *in vitro* for cirrhotic patients. This explains the absence of deoxycholic acid in cirrhotic bile and may be a protective mechanism to prevent formation of the hepatotoxic secondary bile acid, lithocholic acid. Moderate decreases in total biliary secretion of bile acids, phospholipids, and cholesterol have been found in the cirrhotic patients, but large variations in biliary lipid outputs occur in this population. Patients with low deoxycholate also appear to have low cholate secretion rates. No striking abnormality in bilirubin conjugation that might explain the increased incidence of pigmented cholelithiasis in cirrhosis has been identified in the seven patients studied.

Conclusions: Bile composition of patients with alcoholic cirrhosis has been studied and several abnormalities defined. Decreased 7 $\alpha$ C dehydroxylation of primary bile acids may prevent formation of potentially hepatotoxic secondary bile acids. Biliary lipid secretion is reduced. No changes in bilirubin conjugation were seen as a possible explanation for increased pigmented gallstones in this group of patients.

Funds Utilized, FY 76: None

Funding Requirements, FY 77: Anticipated publications costs of approximately \$200.

Publications:

R. G. Knodell, M.D. Kinsey, E. G. Boedeker, and D. P. Collins:  
Deoxycholic acid metabolism in patients with alcoholic cirrhosis.  
*Gastroenterology*, August 1976.

Type of Report: Completed

Work Unit No.: 9009

Title of Project: Abnormalities of B6 metabolism and glycogen metabolism in Hodgkin's disease

Investigators:

Principal: LTC Michael J. Haut, M.D., MC  
MAJ John A. Kark, M.D., MC  
Associate: Johannes Blom, M.D.  
LTC Robert W. Muir, M.D., MC  
MAJ William Babcock, M.D., MC  
MAJ Salvatore Scialla, M.D., MC  
LTC Daniel B. Kimball, M.D., MC  
MAJ A. Richard Miskoff, M.D., MC

Objectives: To evaluate B6 and glycogen metabolism in tissues of patients with Hodgkin's disease in order to answer two questions: (1) Are the diminished levels of vitamin B6 coenzyme in Hodgkin's disease due to alterations in the enzymes regulating B6 metabolism? If so, in what tissues is B6 metabolism altered? (2) Does the deficiency of coenzyme B6 contribute to muscle weakness by decreasing the activity of muscle glycogen phosphorylase, a B6-containing enzyme?

Technical Approach: Our initial studies showed that some patients with Hodgkin's disease or other malignancies had lower plasma B6 levels than control subjects, but had increased capability for red cell conversion of precursors to pyridoxal-5-phosphate under optimal conditions. In order to explain this discrepancy, we have concentrated our efforts this year on examination of B6 metabolism by (1) isolated subpopulations of peripheral blood cells, (2) bone marrow precursors of each type of hematopoietic cell, and (3) specific tissues, particularly lymph nodes, liver, spleen, and muscle.

(1) Isolation of subpopulations of peripheral blood cells. During the past year, methods have been set up to obtain virtually pure populations of erythrocytes, platelets, granulocytes, monocytes, and lymphocytes. Specific methods that have been used include that of Rabinowitz<sup>1</sup> (which involves separation by differential centrifugation followed by passage through a glass bead column) and Boyum<sup>2</sup> as modified by Rickles et al<sup>3</sup> (in which the various types of leukocytes are separated on a Ficoll-Hypaque density gradient).

(2) Isolation of bone marrow precursors of each type of hematopoietic cell. Our attempts during the early part of FY 76 to isolate specific subpopulations of bone marrow cells by density gradient centrifugation met with limited success. In order to obtain a higher degree of resolution, we have switched to velocity sedimentation. Our technique is similar to that described by Denton and Arnstein,<sup>4</sup> in which bone marrow spicules are broken up by passage

through needles of successively smaller diameter, incubated with the appropriate reaction mixture (in our case, one containing labeled pyridoxine or pyridoxal) and then separated by unit gravity sedimentation. We have obtained a Staput unit gravity sedimentation apparatus<sup>5</sup> and are now in the process of setting up this method.

(3) Study of B6 metabolism by specific nonhematopoietic tissues. Using animal tissues, we have established appropriate conditions for assay of PLP and pyridoxal kinase in several nonhematopoietic tissues. We have also set up procedures for chromatographic separation and subsequent fluorometric quantitation of the various B6 vitamers from liver.<sup>6</sup> This procedure will be extended to other tissues, both hematopoietic and nonhematopoietic.

Progress and Results. During FY 75, our screening studies indicated that some but not all patients with Hodgkin's disease or other malignancies have lower plasma B6 levels than control subjects, and increased capability for red cell conversion of precursors to pyridoxal-5-phosphate under optimal conditions. Patients with infectious mononucleosis have enzyme and PLP levels intermediate between those of patients with malignancies and controls. PLP levels per cell are not increased in either the RBC's or the lymphocytes, and the red cell levels appear to reflect the plasma levels.

Before studying additional patients, we felt that we should perfect methods for study of (1) isolated peripheral blood cells, (2) isolated marrow hematopoietic cells, and (3) nonhematopoietic tissues. Our efforts on this project during the past year have focused on establishing workable methods for each of the above. Our progress in these areas is detailed in the "Technical Approach" section of this report.

Conclusions: Since we have concentrated on methods development before embarking on the second, more detailed phase of our study, we can make no new conclusions from our work.

Funds Utilized, FY 76 (for this work unit and #9010): \$6400 for purchase of an Aminco-Bowman spectrophotofluorometer.

Funding Requirements, FY 77 (for this work unit and #9010:

Personnel: None

Equipment: Cell counter, with capability for counting erythrocytes and leukocytes. Coulter ZBI, CMS stock no. 360-529, GSA \$7,100 minus 5% (355) = \$6,745.

Justification: All our biochemical data are expressed as concentration per  $10^9$  or  $10^6$  cells. For example, red cell PLP is expressed as ng PLP/ $10^9$  RBC and lymphocyte PLP as ng PLP/ $10^6$  lymphocytes. When we are studying isolated subpopulations, a large number of counts is necessary. Our current cell counter is more than 10 years old and is no longer repairable. We are presently doing this large volume of counts by hand or taking them to other laboratories to run (when possible).

Supplies: None

Travel: \$300 for presentation of data at one meeting

Other: None

Publications: None

Type of Report: Interim

References:

1. Y. Rabinowitz: Separation of lymphocytes, polymorphonuclear leukocytes and monocytes on glass columns, including tissue culture observations. Blood 23: 811-828, 1964
2. A. Boyum: Isolation of mononuclear cells and granulocytes from human blood. Scand J Lab Clin Invest Suppl 97 (21): 77-89, 1968
3. F. R. Rickles, J. A. Hardin, F. A. Pitlick, L. W. Hoyer, and M. E. Conrad: Tissue factor activity in lymphocyte cultures from normal individuals and patients with hemophilia A. J Clin Invest 52: 1427-1434, 1973
4. M. J. Denton and H. R. V. Arnstein: Characterization of developing adult mammalian erythroid cells separated by velocity sedimentation. Br J Haematol 24: 7-17, 1973
5. Staphut Cell Separator, manufactured by O. H. Johns Scientific Company, Toronto, Canada
6. Y. H. Loo and W. M. Cort: Assay of pyridoxal and its derivatives. In Methods of Neurochemistry, vol 2, 1972, pp 169-204

Work Unit No.: 9010

Title of Project: Vitamin B6 metabolism in the hematopoietic system of patients receiving isoniazid and patients with sideroblastic anemia

Investigators:

Principal: MAJ John A. Kark, MD, MC  
LTC Michael J. Haut, MD, MC  
Associate: MAJ William Babcock, MD, MC  
MAJ Salvatore Scialla, MD, MC

Objectives: To apply new information about the metabolism of vitamin B6 by erythroid cells to the diagnosis and management of toxic side effects of INH and the sideroblastic anemias, including those secondary to malignancies.

Part I, Inhibition of pyridoxal kinase in red cells of patients receiving isoniazid

Technical Approach, Part I: To determine how INH affects PLP metabolism in vivo, we measured activities of the enzymes involved in synthesis and degradation of PLP in red cells of 9 tuberculin-skin-test-converters taking prophylactic INH and 10 controls. Plasma PLP and INH levels were determined on the same subjects. Erythrocyte pyridoxal kinase activity was compared between 5 Caucasians taking INH and 17 Caucasian controls; an identical comparison was made between 5 Blacks and 10 Black controls. This was done because of the known racial difference in pyridoxal kinase activity. To avoid the effect of individual differences in this enzyme activity, a sequential comparison was made comparing the pretreatment erythrocyte pyridoxal kinase activity of 17 subjects with their activity during the second to fourth month of prophylactic INH.

Progress and Results, Part I: Mean pyridoxal kinase activity was 30% lower, while red cell pyridoxine phosphate and B6 phosphatase activity were unchanged in the original 9 subjects of mixed racial composition who were taking INH. In the racially matched groups, those taking INH had 25% lower erythrocyte pyridoxal kinase activity, but owing to marked individual differences in activity, no statistical significance could be shown for this difference. In the sequential study, 16 of 17 subjects taking INH had lower activity of red cell pyridoxal kinase, while 17 controls had no significant change. Using the Wilcoxon signed rank sum test or the Student's t test for paired differences, this effect of INH was highly significant ( $p$  less than 0.001 for subjects taking INH, versus  $p$  less than 0.4 to 0.49 for controls).

Conclusions, Part I: We conclude that (1) treatment with INH causes decreased pyridoxal kinase activity in red cells, but does not affect the red cell PNP-oxidase or B6-phosphatase activities; (2) this supports in vitro evidence that INH inhibits brain and hepatic pyridoxal kinases; (3) a major part of the mechanism by which INH causes B6-responsive side effects is by interfering with synthesis of pyridoxal-phosphate from the nonphosphorylated vitamers of B6. This work encourages one to perform studies designed to identify an inhibitor of pyridoxal kinase in vivo during treatment with INH (see protocol 1647).

Publications, Part I:

M. J. Haut, J. A. Kark, C. T. McQuilkin, T. P. Gibson, and C. U. Hicks: Inhibition of pyridoxal kinase in red cells of patients receiving isoniazid. Presented at Third Meeting of the International Society of Haematology, European and African Division, London, England, August 1975, and published in Proceedings.

J. A. Kark, M. J. Haut, C. U. Hicks, et al: Fluorometric assay of erythrocyte pyridoxal kinase activity. Submitted to J Lab Clin Med.

J. A. Kark, M. J. Haut, C. U. Hicks, et al: Inhibition of erythrocyte pyridoxal kinase in patients taking isoniazid. Submitted to J Lab Clin Med.

Part II, Intrinsic abnormalities of red cell B6 metabolism in the sideroblastic anemias

Technical Approach, Part II: Synthesis of pyridoxal-phosphate (PLP) from pyridoxal or pyridoxine and degradation of PLP by B6-phosphatase were measured in 26 patients with sideroblastic anemia and in 26 controls. Plasma PLP, erythrocyte PLP, and in some cases lymphocyte PLP were measured in these patients concurrently. Patients previously studied are being followed to determine the effects of treatment on B6 metabolism. Studies are underway to develop methods for heme synthesis and B6 metabolism in erythroid cells from the bone marrow. Studies are in progress with Dr. Kenneth Goldstein, Assistant Professor of Medicine, George Washington University School of Medicine, to measure the in vitro effect of drugs on erythroid proliferation of cells obtained from the bone marrow of patients with sideroblastic anemia.

Progress and Results, Part II: Although nearly all patients with untreated sideroblastic anemia had very low plasma PLP concentrations, their red cell PLP concentrations and red cell pyridoxal kinase activities were almost never low. All patients given pyridoxine in

pharmacologic amounts were capable of elevating the red cell PLP and the red cell pyridoxal kinase activity to levels higher than normal. None of our patients showed a significant response to pyridoxine.

Conclusions, Part II: Our data indicate that the basic metabolic lesion preventing heme synthesis is not inability to synthesize erythrocyte PLP unless (1) the circulating red cells are significantly different from those red cells that die in the bone marrow, or (2) the kinetics of pyridoxal kinase or one of the other enzymes involved in B6 metabolism are abnormal. These questions are being addressed by study of isolated marrow erythroid precursors (see report for WU 9009), kinetic study of pyridoxal kinase from normal subjects and from patients with sideroblastic anemia. In addition, heme synthesis by marrow cells from sideroblastic patients is being studied to investigate the strong possibility that the etiology of most of these anemias is an alteration in the heme synthetic pathway.

Publications, Part II:

J. A. Kark, C. U. Hicks, R. Campbell: Kinetics of erythrocyte pyridoxal kinase in refractory sideroblastic anemia. Clin Res 23: 582a, 1975

J. A. Kark, M. J. Haut, T. P. Duffy, C. T. McQuilkin, and C. U. Hicks: Intrinsic abnormalities of red cell metabolism of vitamin B6 in sideroblastic anemia. Presented at Third Meeting of the International Society of Haematology, European and African Division, London, England, August 1975, and published in Proceedings

Funds Utilized, FY 76 (Parts I and II): For this work unit and also #9009, \$6400 was used for purchase of an Aminco-Bowman spectrophotofluorometer.

Funding Requirements, FY 77 (for this work unit and #9009):

Personnel: None

Equipment: Cell counter with capability for counting erythrocytes and leukocytes. Coulter ZBI, CMS stock no. 360-529, GSA price \$7,100 minus 5% (355) = \$6,745. Justification: All our biochemical data are expressed as concentration per  $10^9$  or  $10^6$  cells. For example, red cell PLP is expressed as ng PLP/ $10^9$  RBC and lymphocyte PLP as ng PLP/ $10^6$  lymphocytes. When we are studying isolated subpopulations, a large number of counts are necessary. Our current cell counter is more than 10 years old and is no longer repairable. We are presently doing this large volume of counts by hand or taking them to other laboratories to run (when possible).

Supplies: None

Travel: None (data has already been presented at both national and international meetings).

Other: None

Type of Report (Parts I and II): Interim

Work Unit No.: 9011

Title of Project: Lectin receptors, lymphocyte function and lymphocyte-monocyte interaction in Hodgkin's disease

Investigators:

Principal: MAJ Stephen F. Speckart, M.D., MC  
Associate: MAJ Jeffrey L. Berenberg, M.D., MC

Objectives: Hodgkin's disease represents an immunodeficient state with functional abnormalities of peripheral lymphocytes. Recent evidence supports the presence of lymphocyte surface structural alterations as well. This experiment is designed to further clarify abnormalities in lymphocyte function and structure in this disease.

Technical Approach:

A. Control donors. (1) Peripheral lymphocytes and monocytes from 15 healthy donors will be used as control studies. (2) Normal lymph node lymphocytes from 10 surgical patients will be used as controls in the indicated studies. These patients will be free from cancer, infection, autoimmune, allergic, or collagen diseases, or any other condition known to alter lymphocyte function.

B. Patient selection. A proposed 20 to 30 patients should be studied. Untreated patients are the most desirable; however, those receiving no form of therapy and in a state of remission for at least 12 months could be included. All patients should be carefully staged according to standard procedure, and it is hoped that several patients in each clinical stage will be represented.

C. Patient tissue donation. 100 cc of blood will be drawn from each patient. In cases of significant leukopenia, more blood may be needed. Also needed are fresh diseased lymph nodes from 10-15 patients obtained at staging laparotomy. Drs. Haut and Kark of Dept of Hematology, WRAIR will use the red cells obtained from this donation for their B6 metabolism studies (protocol previously approved), thereby not requiring more blood from these patients than already approved.

D. Laboratory studies. Lymphocytes from peripheral blood and lymph nodes in both patients and control subjects will be subjected to the following experiments:

1. Binding experiments with five lectins: Con-A, E-PHA, L-PHA, Ricinus Communis Agglutinin (RCA-1), and WGA. In these studies lymphocytes are incubated with radio-iodinated lectins and saturable binding curves are plotted. Appropriate calculations are used to determine the number of receptors per cell for a given lectin.

2. In vitro PHA responsiveness: This will include 7-day incubations of lymphocytes with five separate concentrations of E-PHA. Reactivity will be measured by thymidine incorporation. Peak responsiveness will be determined as both a function of incubation time and E-PHA concentration.

3. Cytotoxicity: Lymphocytes will be incubated with  $\text{Cr}^{51}$ -labeled chicken erythrocytes to determine relative cytotoxicity to the erythrocytes as measured by chromium release.

4. Lymphocyte-monocyte interaction studies: See below.

Monocytes from peripheral blood in both patients and control subjects will be subjected to the following experiments:

5. Monocyte chemotaxis: This assay of monocyte function will be used to monitor two test systems in these experiments: (a) to evaluate monocyte responsiveness to known chemotactic factors such as C5a and (b) to test the elaboration of the lymphocyte chemotactic factor by the test lymphocytes. Here the removal of patient monocytes and addition of control monocytes to the lymphocyte cultures will be utilized whenever the lymphocytes appear to be defective.

6. Lymphocyte cultures on monolayers of patient and control monocytes: These studies are designed to evaluate both monocyte chemotaxis just mentioned and the effect of control and Hodgkin's monocytes on the lymphoproliferative response to PHA.

7. Macrophage culture: Lymphocyte culture supernatants will be examined in macrophage culture for their effect on the following functions of macrophages: phagocytosis, adhesion, glucose metabolism, and  $\text{Cl}^{14}$  glucosamine uptake.

Progress and Results: Unfortunately we have not yet had time to work on this project. However, the enthusiasm for this research remains, and our current plan is to begin this project by July 1976.

Conclusions: See above.

Funds Utilized, FY 76: None

Funding Requirements, FY 77: Funding will come from general laboratory funds, which are in the range of \$10,000 per year.

Publications: None.

Type of Report: Interim

Work Unit No.: 9031

Title of Project: Continuous Electrocardiographic Monitoring of Myocardial Infarction Patient

Investigator: Margaret L. O'Dell, LTC, ANC  
186-22-2663  
Nursing Research Service, WRAMC

Objectives:

1. To identify the circadian and ultrarhythms of heart rate in the myocardial infarction patient.
2. To determine if there are detectable factors which alter these rhythms.

Technical Approach:

The heart rate of the subjects is continuously recorded for the period of time spent in the cardiac care unit. A Schwarzer Oszilloscript Polygraph picks up the electrical signal emitted from the activity of the patient's heart and transmits it to a Tandenberg Instrumentation Series 100.

Progress and Results:

Pilot phase consisting of four subjects has been completed. Data not analyzed because of the inability of Department of Experimental Psychophysiology, WRAIR, to perform the required time series analysis.

Termination:

Study terminated as investigator is retiring from active duty as of 31 July 1976.

Work Unit No.: 9033

Title: Nurses' Perceptions of Satisfying and Dissatisfying Factors Affecting Their Ability to Render Quality Patient Care

Date: 12 September 1975

Investigator: Margaret L. O'Dell, LTC, ANC, 186-22-2663, Director, Division of Nursing, WRAIR

Progress & Results: Twenty-four registered nurses, three from each military and civilian grade level (except top level military position) ranging in age from 21 to 65 years, were the subjects. Demographic data was collected on each participant. Other data was collected through unstructured tape recorded interviews. Thematic analysis was utilized to analyze the responses obtained from the nurses. Seven categories were established from the interview materials. These included: administration, communication, education, feedback, interpersonal relations, resources, and utilization. The "satisfying" and "dissatisfying" factors in each category were analyzed quantitatively and qualitatively to identify trends.

All categories except two, education and feedback, demonstrated more dissatisfaction than satisfaction. Differences developed between nurses working in different ward areas. In the small specialized units, such as intensive care, recovery room, and renal dialysis, the nurses voiced more satisfaction than dissatisfaction. Nurses working on the larger ward areas showed more dissatisfaction. In the small specialized units, nurses stated they felt they were treated as "fellow professionals", were given status comparable to other disciplines, and were recognized for the expertise contributed to the health care team. Teamwork was described as central to these small specialized units. Nurses working in the larger ward units, for the most part, did not feel they were treated as "fellow professionals", especially in their relationships with physicians. Communications and interpersonal relations were reported to be poor. The most frequent perceived dissatisfaction was the ability to feel part of a team effort with the physicians. The content presented showed that nurses perceived physicians as seeing them differently, dependent upon whether they work in a small specialized unit or in a larger ward unit.

The shortage of nursing personnel, in general, was frequently mentioned, specifically qualified personnel. The nurses had to work overtime and were often called in to work on their days off because of personnel shortages. Personnel scheduled for duty especially over weekends, were calling in sick, an event that usually occurs when only a minimal work force is present for duty.

In the "administrative" category, three satisfactions were reported as compared to 21 "dissatisfactions". The majority of negative responses indicated that nurses felt that they had too many administrative responsibilities which took them away from their patients. They saw themselves as bogged down in paperwork because there were not enough ward clerks and a number of untrained secretaries.

The improper utilization of personnel generated dissatisfaction as individuals did not feel "fulfilled" in their positions. It was reported that skilled non-professional personnel were being utilized mostly for transporting patients and as messengers. Some stated that this was a factor for their seeking work in intensive care units where they would not be required to leave the clinical setting to do other jobs.

Educational opportunities were seen as satisfactory. In-service programs were being conducted which were meaningful for the nursing staff. For example, a nurse on the dialysis unit reported that ongoing sensitivity meetings focused on patients' needs were more advantageous for the staff.

Having a one-to-one relationship with adequate time to teach patients about their conditions was reported as an asset by a nurse clinician. She also stated that receiving appreciation from patients for her efforts was a source of satisfaction. The fact that she was not fully performing in the role which her education was presumed to have prepared her was frustrating. The younger physicians were more receptive to her than the older physicians. Many physicians simply did not know what she was supposed to do.

Conclusions:

The findings demonstrate that problem areas are seen as existing in this health care facility, which act as deterrents to nurses in their ability to render quality care. The nurses working in the small specialized units paint a picture of satisfaction except for their administrative workload. However, the

nurses working in the larger ward units, for the most part, are dissatisfied as they are not able to render "quality patient care". When life and death is an issue, as in intensive care units, recovery room, and renal dialysis unit, the physicians rely on the nurses as co-professionals but this is not the case in the larger ward units. Here we see the more traditional behavioral patterns operating between nurses and physicians.

One problem seen is a lack of nurses and support personnel to accomplish their goal. Insufficient inputs into a system act as constraints to meeting the goals of the system. Where there is only one nurse on a shift as it presented from the data obtained, the nurse must orient to performing those functions which keep the ward operational. The absence of ward managers, ward clerks, and trained secretaries in the ward areas contribute to the problems encountered. The utilization of skilled nonprofessionals for transportation of patients and as messengers also contribute to the problems.

Type of Report: Completed

Work Unit No: 9052

Title of Project: Disordered Behavior and the Ongoing Family Milieu

Investigators:

Principal: Harry C. Holloway, COL, MC

Associate: John H. Newby, Jr., MAJ, MSC  
Donald R. Bardill, LTC, MSC  
Gloria Setti, MSW  
Rosemary Diliberto, MSW  
Eugene Grossman, CPT, MSC  
T. Peter Bridge, MAJ, MSC  
Joseph M. Rothberg, Ph.D.  
Alberto Rivas, SP5  
Steve Camp, SP5

Objectives: To identify and measure meaningful variables within the family milieu in terms of (1) the nature of the troubled behavior exhibited by an identified patient referred to a child guidance clinic and (2) the identification of factors in the family relevant to the behavior of the identified patient and other family members.

Technical Approach: Structured and semi-structured interviews with selected family members.

Progress and Results: During the period of this report the data collection phase of the study was completed. Data are currently being analyzed with a few toward a project completion date of 30 August 76.

Conclusions: 1. Family members vary in their description of problems being encountered by the family. However, a majority of the problem descriptions revolved around (a) relationships, i.e., parent-child sibling and/or peer and, (b) behavioral manifestation described as delinquent or withdrawal from persons and situations. In an effort to resolve problems family members tend to move toward, away from, or against persons within the family demonstrating the problem behaviors.

2. The data indicate a general lack of confluence between the social network contacts of parents. In this context, parents tend to go their separate ways in relation to leisure-time pursuits outside of the family.
3. In all families in which the identified patient was a female, family members reported a higher percentage of time spent alone than was reported by families in which the identified patient was a male. This data seem to suggest a high level of "perceived isolation" in the families with female patients. The data further suggest that families isolate themselves in different parts of the home and spend little time together as a family. When families engage in a mutually shared activity such as watching television, family members report a limited amount of interaction with each other.
4. In terms of "life stress" indicators, or stressful events experienced by families, children tended to report a significantly higher number of events than were reported by parents. In conjunction with this finding identified patients reported a higher percentage of stressful events than were reported by other family members.

Funds Utilized:	FY-76 None
Funding Requested:	FY-77 None
Publications:	FY-76 None
Type of Report:	Interim

Work Unit No.: 9053

Title of Project: Effects of Treatment on Social Adjustment  
in Patients with Chronic Renal Failure

Investigators:

Principal: Harry C. Holloway, M.D., COL, MC  
Associate: David H. Marlowe, Ph.D.  
T. Peter Bridge, M.D., MAJ, MC  
Everett Spees, M.D., COL, MC

Objectives: The intent of the proposed research is to examine certain interacting co-determinants of outcome of stressful life experience. Patients with chronic renal failure, undergoing either hemodialysis or renal transplantation, have been chosen for this study. These patients are seen as paradigms for individuals exposed to highly stressful life situations. The specific objectives are threefold: (1) To examine the social support systems available to patients with chronic renal failure, to their families, and to those kin who choose to become kidney donors when they are referred to WRAMC for pre-admission or admission evaluation, and to follow prospectively the extent to which the availability of such support systems changes over the course of treatment; (2) to examine symptomatic expression of stress in this population over the course of treatment; and (3) to evaluate the subject's capacity to cope effectively with stress (defensive style/reserve) and to follow prospectively variations in this capacity.

Technical Approach: Research volunteers from the Renal Hemodialysis and Transplant Service, WRAMC, and their spouses (or another member of their family) will be assessed during either the pre-dialysis hospitalization or the hospitalization when hemodialysis is initiated. This assessment includes structured interviews to assess social adjustment, social networks, recent events, and affect in addition to a non-structured interview to assess defensive reserve. Repeat evaluations will take place every 4-6 months for one year after this initial evaluation. Similar evaluations will also be completed for matched non-medically ill volunteers from the WRAMC staff.

Progress & Results: Work on this project was initiated following final approval at WRAIR in January, 1976. All new admissions to the Hemodialysis Unit (Ward 38) at WRAMC were contacted to request their participation in this project. To date a total of 5 volunteers with chronic renal failure and their families have completed their initial evaluations. This rate is less than would have anticipated at this point and appears to be due to the following two reasons: (a) lower patient census on Ward 38 apparently secondary to a decision by the Department of the Army to refer some of the chronic renal failure patients to centers other than WRAMC; and (b) openly expressed reluctance of some patients or their kin to participate in psychiatric research sponsored by the U.S. Army during the time when national press coverage was being given to the Army's earlier experiments with LSD.

Conclusions: Although the acquisition of research volunteers is proceeding at a rate that is slower than would have been anticipated, those who have completed the project to date have provided adequate and useful information for the interviews. Thus it would appear that the choice of instruments and aspects of the proposed methodology other than acquisition of subjects are satisfactory.

Funds Utilized, FY-76: Funding provided by R&D Command

Funding Requirements, FY-77: Funding provided by R&D Command

Publications: None to date

Type of Report: Interim

Work Unit No.: 9079

Title of Project: Effects of a Structured Audio-Visual Preoperative Teaching Approach on the Incidence of Atelectasis in Chronic Renal Failure Patients Undergoing Abdominal Surgery

Investigators:

Principal: Kathleen Srsic, Cpt. ANC

Associate: Valerie Olszewski, Cpt. AMSC  
Molly Bugenhagen, Lt. ANC

Objective: The objective of this study is to determine if there is a relationship between the type of structured preoperative approaches conducted and the incidence of atelectasis in chronic renal failure patients undergoing abdominal surgery.  
This study has been undertaken to complete the thesis requirements for a master's of science degree.

Technical Approach:

Setting: Ward 38, Renal Unit at WRAMC

Sample: 1) chronic renal failure patients undergoing abdominal surgery  
2) 18 years of age or older  
3) have understanding and command of the English language  
4) are capable of performing protocol tasks  
5) have a physician's order for IPPB treatments postoperatively  
6) are able to sign an informed consent  
7) have a physician's written consent for participation in the study

Design: The original design intended was an experimental pre-test post-test control group and post-test only control group.

Method: The experimental group patients receive a structured audio-visual preoperative teaching approach within 48 hours before surgery. Demonstration and return demonstration of postoperative exercises is required. Control group patients receive a structured pre-operative teaching approach within 48 hours before surgery. Demonstration and return demonstration of postoperative exercises is required. Content for both groups is the same however, the control group patients receive no audio-visual media supplements.  
Both groups have preoperative and postoperative

chest x-rays taken; vital capacities measured within 48 hours preoperatively and 24, 48, and 72 hours postoperatively; and a record maintained for the number of times that nasal-tracheal suctioning is required.

**Progress and Results:** Only one patient has met the criteria for inclusion in this study. This patient was placed in the experimental group. No postoperative complications developed with this patient.

Another patient is scheduled for surgery before the end of this month and will be placed in the control group upon her consent and her physician's consent for participation.

**Conclusions:** A true experimental study with an adequate sample size is not possible due to the limited number of surgical procedures performed on chronic renal failure patients. This has been discussed with faculty advisors and a case study analysis will be completed in lieu of the original intent for data analysis. The month of August has been set for the termination of the data collection period.

**Funds Utilized, FY-76:** None

**Funding Requirements, FY-77:** None

**Publications:** None

**Type of Report:** Interim

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DEPARTMENT OF THE ARMY  
HEADQUARTERS WALTER REED ARMY MEDICAL CENTER  
Washington, D.C. 20012

WRAMC Regulation

1 April 1973

Clinical Investigation Program

WRAMC RESEARCH ACTIVITIES

	Paragraph
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1. PURPOSE. This regulation prescribes the policies and procedures applicable to the Clinical Investigation Program within the Patient Care Facility at Walter Reed Army Medical Center.

2. CRITERIA. Clinical investigation activities will meet the following criteria:

a. The objectives have scientific merit and are reasonably attainable.

b. The investigators are competent to perform the studies proposed.

c. Resources required for the proposed studies are either available, or can be obtained, and are proportionate to the merit of the proposal.

d. The studies will not have a deleterious effect upon the care of the sick and wounded.

e. The studies are performed in a considered, coordinated, and professional manner.

\*This regulation supersedes HR 70-1, 9 December 1971.

1 April 1973

f. The rights, well-being, and dignity of human subjects are maintained in accordance with the principles of the Declaration of Helsinki of the World Medical Association, and that written consent is obtained when indicated.

g. Any research involving animals will conform with AR 70-18 and the Laboratory Animal Welfare Act (Public Law 89-544; 7 USC 2131 et seq).

h. Assure compliance with existent military regulations to include AR 40-7, Use of Investigational Drugs in Humans; AR 40-37, Radioisotope License Program (Human Use); AR 70-25, Use of Volunteers as Subjects of Research; and WRAMC Reg 40-10, Health Physics Regulation.

i. The voluntary consent of the human subject is essential. Each individual who initiates or directs the clinical investigation has a personal duty and responsibility for ascertaining the quality of the subject's consent. Before the acceptance of the subject, he must be given adequate explanation. He must be informed of the nature, duration and purpose of the study; the methods and means by which it is to be conducted; all inconveniences and hazards reasonably to be expected; and the effects upon his health or person which may possibly come from his participation in the study. Written consent will be obtained in accordance with the format outlined in the appendix and will be in nonmedical language that is easily understood by the subject.

### 3. DEFINITIONS.

a. Clinical investigation under this program consists of the organized scientific inquiry, both in humans and by directly related laboratory work, into clinical problems of significant concern in the necessary health care of members of the military community, including active duty personnel, dependents, and retirees.

b. Subjects are any persons who may be at risk because of participation as an object of clinical investigation by members of the AMEDD or their appointed representatives. These may include inpatients, outpatients, organ donors, informants, or normal individuals who participate in studies of medical, physiological, sociological, or psychological orientation.

1 April 1973

WR 70-1

c. At risk: A person is "at risk" if he/she may be exposed to the possibility of harm (physical, psychological, or sociological) as a consequence of activity which extends beyond use of established and accepted methods necessary to meet his/her needs. Determination of nature and extent of "at risk" is a matter of common sense and professional judgment. Responsibility for this determination resides at all levels of institutional and departmental review.

4. COMMITTEES. The following committees will be appointed:

a. Clinical Investigation Committee: To review all clinical investigation proposals for scientific adequacy and to establish priorities for support. For the purpose of recommending new drugs which have not been released by the Food and Drug Administration, the committee will serve also as the Therapeutic Agents Board (para 126, AR 40-2). This committee will be composed of the following:

Director, Professional Services (Chairman)  
Chief, Clinical Investigation Service (Secretary)  
Chief, Department of Medicine  
Chief, Department of Surgery  
Chief, Department of Pathology  
Chief, Department of Radiology  
Chief, Department of Pediatrics  
Director, WRAIR  
Chief, Health Physics  
Chief, Pharmacy Service (ex officio)  
Chief, Patient Administration (ex officio)  
Chief, Nursing Research Service (ex officio)

b. Human Use Committee: To review for medical safety and suitability all clinical investigation protocols involving the use of human subjects. This committee will be composed of the following:

Director, Professional Services (Chairman)  
Chief, Clinical Investigation Service (Secretary)  
A Chaplain  
A JAG Officer  
Chief, Department of Nursing  
Chief, Department of Psychiatry and Neurology  
Chief, Department of Obstetrics and Gynecology  
Command Sergeant Major  
Director, Human Resources Directorate  
Chief, Department of Dentistry  
Clinical Pharmacist, Hematology-Oncology Service

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5. CLINICAL INVESTIGATION COMMITTEE: The Clinical Investigation Committee will meet once monthly, usually on the fourth Tuesday at 1400 hours. The committee will review all new research proposals. Periodically, the committee will review approved and ongoing research. Each project will be reviewed at least once yearly, at the termination of the research and whenever there is a major change either in the goals or the procedures or drugs used in human subjects. Adverse reactions to investigational drugs or procedures will be promptly reported to the committee. The committee will make recommendations to the Commander.

6. HUMAN USE COMMITTEE: The Human Use Committee will meet once monthly, usually on the fourth Tuesday at 1500 hours. The committee will review all new research proposals in which human subjects are used. Periodically, the committee will review approved and ongoing investigational studies in which humans are used. Each project will be reviewed at least once yearly and whenever there is a major change in the goals or the procedures or drugs used in human subjects. The committee will make recommendations to the Commander.

#### 7. RECORDS AND REPORTS.

a. Initial Report. Requests for initiating research projects will be submitted in one copy to the Commander, Walter Reed Army Medical Center, ATTN: Chief, Clinical Investigation Service. This will be submitted by the principal investigator through the chief of the respective service and department, and prepared as described in Appendix A. When radiological, laboratory, or nursing support is required, the principal investigator should have obtained the concurrence of the appropriate chief of service prior to submission to the Clinical Investigation Committee. The chief of the department proposing the study will provide a written forwarding comment describing the study in relationship to its scientific contribution, military relevance, and contribution to the teaching program; an indorsement that the proposal conforms to the criteria described in paragraph 2 above; and the availability or unavailability of the resources requested in the proposal.

b. Annual Progress Reports. Annual progress reports will be prepared for each approved project as prescribed by AR 40-38, Clinical Investigation Program and will be submitted to Clinical Investigation Service prior to 15 May of each year until the investigation is completed. See Appendix B.

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c. Interim Reports. Interim reports are discretionary and may be submitted at any time when important development, adversities or other circumstances occur which should be brought to the attention of higher headquarters. Interim reports may also be used to add or remove procedures or methods from the original protocol.

d. Final Reports. Final reports are required upon completion or termination of a specific research effort. The report will include a summary of all work performed, results obtained, together with copies of all publications, whether printed, in press or submitted for publication. Inclusion of references to previous progress reports is optional. If the project is terminated prior to completion, the reasons for termination should be reported. Report is due within 30 days following completion or termination of effort.

e. Special Therapeutic or Diagnostic Procedures. Any special therapeutic or diagnostic procedures or any new, hazardous, or otherwise noteworthy therapeutic or diagnostic measures will be recorded in Space 24 of DA Form 8-274, Clinical Record Cover Sheet for Inpatients.

f. All reports will be forwarded to the Clinical Investigation Service following review by the appropriate chief of service and department. The Clinical Investigation Service will schedule presentations to the appropriate hospital review committees. Following review by the commander of committee reports the Clinical Investigation Service will insure that reports are forwarded to the Surgeon General as required by AR 40-38.

8. REPORTS TO PHARMACEUTICAL COMPANIES. For procurement of investigational drugs which have not yet been released by the Food and Drug Administration, detailed reports to the drug company are required by FDA (Form FD 1573). The reports are the responsibility of the principal investigator, and are a matter of direct communication between him and the drug company.

9. REQUEST FOR FUNDS. Requests for funds to support clinical investigation program are presented to the Center Command annually during the month of March.

a. Projects requiring refunding in the amount of \$1,000.00 or more are submitted each year prior to 1 March in the format of Appendix A for consideration.

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b. New proposals which require funds may be submitted at any time. Approval of funding is dependent upon availability of local or Surgeon General resources. Format Appendix A.

10. INFORMED CONSENT:

a. Patient Consent. The utilization of drugs or procedures which have not yet been accepted or established by common use require the patient's consent. The patient must be informed, i.e., his consent must be based upon his having knowledge of the experimental nature, purpose, and possible hazards. The consent should be in writing, except as provided in paragraph 7b, AR 40-7.

b. Human Volunteer. Investigative studies in which drugs or procedures are employed that will not benefit the person are subject to, and must comply with AR 40-7, Use of Investigational Drugs and/or AR 70-25, Use of Volunteers as Subjects of Research in addition to AR 40-38.

HSW-QCCR

FOR THE COMMANDER:

FRED C. BRAND  
LTC, MSC  
Adjutant

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I plus 100 cys to  
Clinical Investigation  
Service

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APPENDIX A

APPLICATION FOR CLINICAL INVESTIGATION PROJECT

1. PRINCIPAL INVESTIGATOR:
2. PROJECT TITLE: (Enter short project title.)
3. OBJECTIVE: (Brief but specific statement of the objective of the project.)
4. MEDICAL APPLICATION: (Explain briefly the medical importance and possible usefulness of the project.)
5. STATUS: (What has been accomplished or published in the proposed area of study and in what manner will the project relate to or differ from that which has been accomplished. If references or personal communication with other Army medical facilities are involved, so indicate.)
6. PLAN: (Outline exactly what is proposed to be accomplished in sufficient detail to indicate a clear course of action. Technological validity of procedures and chronological steps should be shown.) (NOTE: The Surgeon General and the local commander must have a very clear picture of how the investigation will proceed to meet the objective of the project. This paragraph frequently furnishes the basis for approval or disapproval of a project.)
7. BIBLIOGRAPHY: (List source of information.)
8. FACILITIES TO BE USED: (Such as laboratory, ward, clinic.)
9. TIME REQUIRED TO COMPLETE: (Give month and year of expected start and anticipated completion.)
10. PERSONNEL TO CONDUCT PROJECT: (List names and positions of persons to be directly involved in project work.) (Attach short biographical sketch, including resume of education, research training, and list of publications, for each person named.)

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11. FUNDING IMPLICATIONS:

	O&MA	OPA	TOTAL
a. Personnel: (itemize and explain need)	\$ ____	\$ ____	\$ ____
b. Equipment: (itemize and explain need)	\$ ____	\$ ____	\$ ____
c. Consumable Supplies: (itemize)	\$ ____	\$ ____	\$ ____
d. Travel: (itemize and explain need)	\$ ____	\$ ____	\$ ____
e. Modification of Facilities: (explain)	\$ ____	\$ ____	\$ ____
f. Other (explain)	\$ ____	\$ ____	\$ ____
TOTAL	\$ ____	\$ ____	\$ ____

12. DATE PREPARED: (Give day, month and year of preparation.)

(Signature of Principal Investigator)

(Signature of Department Chief)

(Enter title and mailing address of Principal Investigator)

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APPENDIX A  
IMPACT STATEMENT

Patients:

Bed Occupancy:

Laboratory:

Radiology:

Pharmacy:

Nursing Service:

Registrar:

Other:

Approvals   Chief of Service   Chief of Dept   For Hosp Comm

Date:

Sig:

Name:

Grade:

Position:

R & D Approval Requested    Yes    No   Date:

Yes    No   Date:

{This is a format. It will not be used as a form.)

A-3

APPENDIX A  
VOLUNTEER AGREEMENT

3 November 1976

I, \_\_\_\_\_, having attained my eighteenth (18th) birthday, and otherwise having full capacity to consent, do hereby volunteer to participate in an investigational study entitled:

---

---

---

\_\_\_\_\_, under the direction of \_\_\_\_\_  
of the Department/Division/Institute of \_\_\_\_\_  
Walter Reed Army Medical Center, Wash., D. C.

The implications of my voluntary participation; the nature, duration and purpose of the study; the methods and means by which the study is to be conducted; and the known inconveniences and hazards have been thoroughly explained to me by the principal investigator or by one of the coinvestigators and such inconveniences and hazards are set forth in detail on the reverse side of this Agreement, along with my initials or signature. I have been given an opportunity to ask questions concerning this investigational study and my participation in the study, and any such questions have been answered to my full and complete satisfaction.

During the course of my treatment as a patient at Walter Reed Army Medical Center I have been provided with a copy of a Privacy Act statement (DD Form 2005) which has made me aware of the safeguards available to me because of the Privacy Act of 1974. I have been given the opportunity to review the DD Form 2005, ask questions and to retain a personal copy. I have been made aware that the information gained about me, because of my participation in this investigational study, may be publicized in medical literature, discussed as an educational model, and used generally in the furtherance of medical science. I freely consent to provide such personal information as is requested of me for this investigational study and freely consent to the disclosure of pertinent personal information derived from my participation in this investigational study for reasons of publication in medical literature, discussion as an educational model and for those additional reasons which specifically relate to the furtherance of medical science.

I am aware that at any time during the course of this investigational study I may revoke my consent and withdraw from this study, without prejudice; however, I may be required for medical reasons to undergo further examinations if in the opinion of my attending physician such examinations are necessary for my health or well being.

---

Signature

Date

I was present during the explanation referred to above, as well as during the Volunteer's opportunity to ask questions. I hereby witness the Volunteer's signature.

---

Signature

Date

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On this side of the Volunteer Agreement, the principal investigator should set forth full details concerning the investigation study, insofar as such would affect or influence the tentative subject in any way. This explanation should be worded so that it can be clearly understood by the subject. The subject should place his initials at the end of the last line of explanation.

A proper explanation should, at a minimum, provide the answers to the following questions:

1. What will be administered or done to the subject?
2. How long will the subject's participation last?
3. To what tests or examinations will the subject be required to submit?
4. Why is the investigation being conducted?
5. Has this particular study been done previously, and, if so, with what results?
6. What inconveniences or discomforts is the subject likely to experience?
7. What risks or hazards can be reasonably anticipated?
8. What steps will be taken to prevent or minimize these risks or hazards?

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APPENDIX B

Annual Progress Report FY\_\_

Work Unit No.:

Title of Project:

Investigators:

Principal: (senior investigator responsible for project)

Associate: (coinvestigators)

Objectives: (goal of research)

Technical Approach: (method of attaining objectives)

Progress & Results: (organized description of the research effort in relation to this work unit which was performed during the period of this report. If investigational drugs were used the information required by AR 40-7 must be included)

Conclusions: (concise statement of goals achieved by current studies)

Funding Requirements: (present and next FY)

Personnel: (name and grade)

Equipment:

Supplies:

Travel:

Other:

Publications: (list only those published during present FY from your service which are related to the research described in this report)

(Report should be typed on 8 x 10-1/2" bond paper with 1" margins on all four sides. Do not number pages. Double space between sections of the report. Single space typing within each section.)

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